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SOME FACTORS AFFECTING THE TRANSMISSION OF LEAF-ROLL VIRUS BY APHIDS

BY B. KASSANIS

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(With Plate 9)

Datura tatula is a more suitable host than potato for studying the factors influencing the transmission of potato leaf-roll virus by *Myzus persicae*; it is more easily infected, provides a better source of virus for feeding aphids, produces symptoms more quickly and over a longer period of the year.

Loughnane's (1943) claim that leaf-roll virus is transmitted by starved aphids that feed for only 5 min. on infected potato plants was not confirmed. The shortest infection-feeding time in which *M. persicae* aphids became infective was 2 hr.; such aphids did not infect healthy plants in the first 2 days and, when transferred to a series of healthy plants at intervals, infected only few. The ability to cause infections was increased by increasing the length of infection feeding. Aphids fed for many days on infected plants could infect healthy plants in the first 15 min. of test feeding, and they continued to cause infections for long periods.

Aphids became infective more readily when feeding on recently infected *Datura tatula*, showing only slight symptoms, than on older plants with pronounced chlorosis; similarly, young potato sprouts showing no symptoms were better sources of virus for aphids than older plants showing severe leaf roll.

The differences in severity of symptoms shown by potato plants with leaf roll in the field mainly occur because of differences in virulence of accompanying strains of potato virus *X*, but isolates of leaf-roll virus were found that also varied in virulence.

Although the literature dealing with potato leaf roll is extensive, there is very little information on the factors that influence its transmission by insect vectors.

The first claim to have transmitted leaf-roll virus by insects was made by Elze (1927), who described four species of aphids and four other kinds of insects as vectors. Since then numerous workers have confirmed its transmission by aphids, of which *Myzus persicae* Sulz. seems to be the most important, but other kinds of insects have not been found to act as vectors. Elze suggested that the virus requires an 'incubation period' of between 24 and 48 hr. in *M. persicae*, for in the few tests he made he found that aphids fed for 14 or 24 hr. on infected plants rarely caused infection in the first day they fed on healthy plants. He also found that aphids could remain infective for a week after feeding on infected plants.

Smith (1929, 1931) made the only detailed study on the relation between leaf-roll virus and its vector *M. persicae*. He showed that the whole process of transmission could be made in 54 hr. The aphids required a minimum of 6 hr. to pick up the virus from infected plants and 2 hr. to transmit it to healthy plants. The rest of the

time was needed for the aphids to develop their ability to infect. He termed this the 'delay period'.

Watson & Roberts (1939) divided the insect-transmitted viruses into persistent and non-persistent types. The vectors of the first group need more than a day feeding on the infected plant to become infective and retain their infectivity for long periods, whereas those of the second group acquire the virus after a few minutes infection feeding and lose their infectivity in a matter of hours. They found that vectors of some non-persistent viruses transmit better if they are starved for a time before the infection feeding. On Smith's results they included potato leaf-roll virus in the persistent group, but this was brought into doubt by Loughnane (1943), who stated that the virus could be transmitted in the manner typical of the non-persistent viruses.

It has been generally assumed that the delay period starts from the time the insects are placed on the infected plants, but Bawden (1943) pointed out that there was no published evidence to substantiate this. In fact there are in the literature two instances where the delay period is apparently unaffected by the length of infection feeding. One is sugar-beet curly-top virus (Freitag, 1936, Table 3) and the other pea enation mosaic virus (Chaudhuri, 1950). Maramorosch (in Bawden, 1950) has now shown that the vector of aster yellows virus, known to have a long delay period, will infect healthy plants the day it leaves the infected plant, provided that it had a long infection feeding. Also, Prentice & Woollcombe (1951) found that the delay period which the aphids require to transmit strawberry virus 3 decreased with increasing infection-feeding time from 10–19 days to 1 day.

This paper describes experiments showing that potato leaf roll has a delay period which starts at the beginning of the infection feeding.

MATERIALS AND METHODS

Dykstra (1933), while investigating common weeds which might harbour potato viruses, transmitted leaf-roll virus by aphids to *Datura tatula* L., *D. stramonium* L. and some other solanaceous plants. Since then, several workers have reported that species of *Datura* and *Physalis* were more susceptible to infection than potato plants. In addition to being easier to infect than potato, *Datura tatula* develops symptoms more speedily and over a longer period in the year and is a better source of virus for aphids. For these reasons it has been largely used in the experiments recorded in this paper.

Interveinal chlorosis usually appears on *D. tatula* 2–3 weeks after infection. This gradually intensifies, and the symptoms show as a green network against a golden yellow background. The leaves become leathery and slightly rolled inwards along the margins. The time taken for the appearance of symptoms depends on the age of the test plants, the season and the method used for infection. It is reduced by increasing the numbers of aphids, by using a good source of virus and by prolonging the length of the infection feeding. *D. tatula* plants are best as sources of infection

when symptoms are only just beginning to appear and, unless otherwise stated, aphids were fed on such plants.

Eight days was the shortest recorded period between inoculation and the appearance of the first symptoms. It occurred with plants about 3–5 in. high, colonized with ten aphids, which had been reared on newly infected *D. tatula*. Symptoms developed much later and were milder than the average in plants colonized with single aphids or with groups which had received short infection feeding or had fed on old infector plants. In one experiment using a poor infector the symptoms were localized on the colonized leaves of the test plants, until the tops were cut off and fertilizers added, when the new leaves developed typical symptoms.

Although experiments with *D. tatula* could be made at times of the year when potatoes would show no symptoms, the *Datura* plants did not react satisfactorily between October and March. Plants infected during this period showed slight stunting but no other symptoms, even when the day length was extended by 4 hr. by illumination with 1000 W. lamps. When used in September the extra light hastened the appearance and intensified the severity of the symptoms, but in mid-winter the plants tended to act as symptomless carriers despite the light treatment. That the virus could be transmitted in winter months was demonstrated by keeping the inoculated plants until the spring, when typical symptoms appeared.

The potato plants used were virus-free stocks of the varieties Majestic, Arran Consul, Craig's Defiance and Up-to-Date. Tubers were sprouted in the dark, and pieces bearing single sprouts were colonized with aphids and placed separately in covered Petri dishes lined with blotting-paper.

At the end of the experiment the aphids were killed by fumigation and the plants kept in insect-proof glasshouses. Symptoms of primary leaf roll were consistently produced in infection experiments made from April to July but could not be accurately recorded in experiments made later in the summer; as a check, tubers were saved from all plants and grown in the next year to record infection.

Myzus persicae were exclusively used and reared on cruciferous plants unless otherwise stated. When they were starved, they were kept in Petri dishes covered with Cellophane.

The term 'transmission period' will be used for the time taken for all the stages necessary for an initially virus-free insect to acquire virus, to develop its ability to infect (delay period), and to infect a healthy plant. The times taken to acquire virus and to infect healthy plants will be called 'acquisition' and 'inoculation period' respectively.

The state of the infector plant

The transmission of leaf-roll virus by aphids that have fed for considerable periods on infectors is reasonably well established. In addition to this method, Loughnane (1943) has claimed transmission by aphids previously starved for 4 hr. and then given only 5 min. infection feeding. I have been unable to confirm this claim.

Table 1 gives the results of six experiments made at different times over the period of 2 years, with the potato varieties Majestic and Arran Consul, and shows that transmission never occurred when aphids were starved and had a short infection feeding.

TABLE 1. *Comparative transmission of leaf-roll virus by aphids*, Myzus persicae, *given long and short infection feeding combined with starving*

Variety ...	Majestic			Arran Consul		
Dates of experiments ...	30. v. 47	7. vi. 47	4. vii. 47	11. v. 48	25. v. 48	9. vi. 48
Aphids* fed on infected Majestic plants for 6 days	9/15†	9/15	0/15	9/10	4/10	3/10
Aphids starved for 4 hr. before feeding on infected Majestic plants for 3–5 min.	0/15	0/15	0/15	0/10	0/10	0/10

* Fifteen aphids were transferred each time on a sprouted tuber, on which they were left to feed for a week.

† The denominator indicates the number of plants colonized, and numerator those which became infected.

In making transmissions from the same potato plants at different times, it was noticed that more success was attained early in the season when the infectors were young than later. This phenomenon can be observed from Table 1, which records the dates of various experiments. Except for the age of the infectors, other factors were, as far as was known, constant in the different experiments. It seemed, therefore, that aphids acquired virus more readily when feeding on young infected plants, which show few or no symptoms, than when feeding on old plants showing unmistakable leaf roll. This idea was confirmed by experiments in which infected potato plants of different ages, but all progenies of the same plant, were used as infector plants (Table 2). Table 3 shows that with *Datura tatula*, also, recently infected plants showing only incipient symptoms are much better sources of virus for aphids than plants infected for some months and showing acute symptoms. Transmission can be obtained from old infected potato and *D. tatula* plants by increasing the numbers of aphids but, as Table 3 shows, differences resulting from the suitability of the infector are detectable up to eight or more aphids per test plant. This effect of increasing age in reducing the availability of leaf-roll virus is not specific to potato plants raised under glass, but was also found in tests using plants from the field as infectors. No definite reason for this effect can be given, but the most plausible explanation is that the virus content of the infected plants reaches an early maximum and then falls. If this is so then the severity of symptoms is not directly related to virus content. However, the results may equally mean that virus is differently distributed in plants infected for different periods, or that the feeding habits of aphids vary with the condition of the plant. A further factor that may be involved in this phenomenon is the occurrence of phloem necrosis. This is pronounced in older potato plants which have well-developed external symptoms. Phloem necrosis

also occurs in infected plants of *D. tatula*, but even in old infected plants the proportion of phloem that appears necrotic when stained with the modified phloroglucinol test (Wilson, 1948) is small when compared with infected potato plants.

TABLE 2. *Transmission of leaf-roll virus from three ages of Arran Consul potatoes to healthy Craig's Defiance, by groups of ten aphids*

Appearance of the infector plants (planted at intervals of 3 weeks)	Infection		
	Exp. 1	Exp. 2	Exp. 3
Plants 2 ft. high, chlorotic and with pronounced leaf rolling	0/9*	0/10	13/20
Plants 1 ft. high, some pallor, slight rolling on bottom leaves	4/9	2/10	.
Plants just appearing through the soil, no symptoms	8/9	4/10	20/20

* The denominator indicates the number of plants colonized, and numerator those which became infected.

TABLE 3. *Influence of the age of infector plant on the transmission of leaf-roll virus by different numbers of aphids*

No. of aphids per plant	From Arran Consul to Craig's Defiance, aphids fed on the same infected plants at		From *Datura tatula* to plants of the same species	
	1. iv. 49	2. v. 49	Aphids fed on newly infected plants	Aphids fed on plants infected for 4 months
1	2/10*	0/10	9/15	0/15
2	3/10	0/10	10/15	0/15
4	4/5	0/5	12/15	3/15
8	5/5	2/5	5/5	3/5
16	5/5	4/5	.	.

* The denominator indicates the number of plants colonized, and numerator those which became infected.

In addition to age the nutritional status of infected plants also affects the readiness with which aphids become infective. With variation in nitrogen, unlike variation in age, suitability as a source of virus is correlated with severity of symptoms. Symptoms in potatoes are masked by application of nitrogen and a smaller proportion of infective aphids is associated with high-nitrogen plants. For example, in one experiment aphids were given an infection feeding of 2 days before being transferred in groups of ten to each of sixteen *D. tatula* plants; the aphids from the low-level nitrogen plants infected six and those from the high-level two.

Similar results were obtained with infected *D. tatula* plants which were given different levels of nitrogen. Fifteen plants which had just started to show symptoms were each given 1 g. ammonium sulphate, and this was repeated a fortnight later, and an equal number of plants were left untreated. A month later the nitrogen-treated plants were large and showed only slight symptoms on the lower leaves,

whereas the controls were stunted, had intense interveinal chlorosis and the older leaves were rolled (Pl. 9, fig. 1). The two groups of plants were colonized with aphids for 2 days and these were then transferred to healthy *D. tatula*, using three aphids per plant, and five infections were obtained from the nitrogen-treated plants and eleven from the untreated out of twenty used for each treatment. The aphids on the high-nitrogen plants fed more continuously and appeared to breed more freely than those on the untreated plants. This suggests that the relative inefficiency in transmitting is more likely to be attributable to a lower virus content in the nitrogen-treated plants than to a distaste for these plants causing a reduction in feeding. Positive interpretations, however, are impossible because there is some evidence that leaf-roll virus is mainly confined to the phloem, and it may be that in the high-nitrogen plants the aphids fed less in the phloem than in the untreated plants.

Acquisition and inoculation periods

Smith (1931) demonstrated that aphids could acquire potato leaf-roll virus from infected potatoes in feeding periods of 6 hr. and, provided they had passed the delay period, could infect healthy plants in feeding periods of 2 hr. Using *D. tatula* both as infector and test plants, both these periods can be reduced. The aphids were fed on the infector plants for periods of 2, 4 and 8 hr. and 1 day and then, in groups of ten, were left for 2 days on the test plants. There were thirty test plants per treatment, and from these 4, 7, 13 and 20 became infected respectively for the four treatments. Testing for the inoculation period, groups of ten aphids, which were fed for 2 days on infector plants, were left for periods of 2, 4, 16 hr. and 1 day on one lot of test plants and for 2 more days on a second lot. Out of ten plants colonized, 1, 6, 5, 8 and 9 became infected. All fifty of the second lot of test plants became infected, showing that every aphid group could produce an infection if left long enough on the test plant.

Although aphids transmitted leaf-roll virus after 2 hr. infection and test feeding, the number of infections increased with increasing feeding time on the infector and test plants. Adult aphids reared on infected *D. tatula* were highly infective. Using such aphids an experiment was made to compare 4 hr. undisturbed test feeding on *D. tatula* with 4 hr. when aphids were disturbed every half hour with a camel-hair brush and with 4 hr. undisturbed feeding after 4 hr. starving. The experiment was repeated three times using two aphids per test plant and thirty test plants in all for each treatment. The total numbers of infected plants were 23, 17 and 23 respectively for the three treatments, showing that neither disturbing nor starving affected transmission, and suggesting that the inoculation period was not more than 30 min. In the next experiment four groups of twenty aphids, reared on infected *D. tatula*, were moved in succession to twenty-two test plants, at intervals of 5, 10, 20 and 40 min. On the first day of the experiment the aphids were moved successively from the 1st to the 11th plant, on which they stayed until the next day, when again

they were moved from the 11th to the 22nd, being left on the last plant for 2 days. The results (Table 4) show that by using very infective aphids the inoculation period could be reduced to 10–15 min., which is also the minimum time (Roberts, 1940) required for the aphids' stylets to reach the phloem. This may be interpreted that potato leaf-roll virus has to be introduced into the phloem for infection to occur.

TABLE 4. *Minimum inoculation period in the transmission of leaf-roll virus to* Datura tatula, *by four groups of twenty aphids reared on infected* Datura tatula

Aphid group	Feeding time on each test plant (min.)	Successive test plants										
		1	2	3	4	5	6	7	8	9	10	11
1	5	−	−	−	−	−	−	−	−	−	−	+
2	10	−	−	−	−	+	−	−	+	−	+	+
3	20	+	+	+	+	+	+	+	+	+	+	+
4	40	+	+	+	+	+	+	+	+	+	+	+
	No. of aphids survived on each transfer	80	80	80	80	79	76	76	76	76	74	73

Aphid group	Feeding time on each test plant (min.)	Successive test plants										
		12	13	14	15	16	17	18	19	20	21	22
1	5	−	−	−	−	−	−	−	−	−	−	+
2	10	+	−	−	−	−	−	−	−	+	−	+
3	20	+	+	+	+	+	+	+	+	+	+	+
4	40	−	+	+	+	+	+	+	+	−	−	+
	No. of aphids survived on each transfer	69	68	68	65	64	64	63	60	58	57	56

Crosses indicate successful transmissions.

INFLUENCE OF THE LENGTH OF INFECTION FEEDING ON THE TRANSMISSION PERIOD

The minimum transmission period for potato leaf-roll virus from potato to potato was found to be approximately 54 hr. (Smith, 1931). Unlike the acquisition and inoculation periods, the transmission period was not much reduced by using *D. tatula* (Table 5). The aphids were fed on the infected *D. tatula* for 17 hr. and then, in groups of ten were tested on a succession of plants, at chosen intervals. Only one out of the twelve groups of aphids transmitted the virus between 45 and 49 hr., five groups between 49 and 65 hr. and the rest in longer periods. These periods include the time the aphids spent on infected plants.

The main purpose of the experiment reported in Table 5 was to get some information as to when the delay period starts. The performance of two lots of aphids, one reared, and the other fed for only 17 hr., on infected *D. tatula* plants, was compared by feeding groups of ten aphids on a succession of test plants for varying intervals. The aphids used for the 17 hr. infection feeding were taken from a colony reared on

healthy *D. tatula* to avoid any effects on feeding habits. All the ten groups of aphids reared on infected plants started infecting in the first 4 hr. of test feeding, while from the twenty groups with 17 hr. infection feeding only twelve produced an infection at all and none before 32 hr. of feeding on test plants.

TABLE 5. *The effect of short and long infection feeding on the infectivity of groups of ten* Myzus persicae *on* Datura tatula

Feeding time on each successive test plant (hr.)	The infectivity of ten groups of aphids reared on infected plants										No. of aphids survived on each transfer
	1	2	3	4	5	6	7	8	9	10	
4	+	+	+	+	+	+	+	+	+	+	100
4	+	+	+	+	+	−	−	+	+	+	100
16	+	+	+	+	+	+	+	+	+	+	100
4	+	+	+	+	+	+	+	+	+	−	99
4	+	+	+	+	+	+	+	+	+	+	99
16	+	+	+	+	+	+	+	+	+	−	99

Feeding time on each successive test plant (hr.)	The infectivity of twelve groups of aphids fed on infected plants for 17 hours												No. of aphids survived on each transfer
	1	2	3	4	5	6	7	8	9	10	11	12	
4	−	−	−	−	−	−	−	−	−	−	−	−	120
4	−	−	−	−	−	−	−	−	−	−	−	−	116
16	−	−	−	−	−	−	−	−	−	−	−	−	111
4	−	−	−	−	−	−	−	−	−	−	−	−	106
4	−	−	−	−	−	−	−	−	−	−	+	−	97
16	−	−	−	+	+	+	+	−	−	−	−	+	95
4	−	−	−	−	+	−	−	−	−	−	−	−	91
4	−	−	−	−	+	−	+	−	−	−	−	−	85
48	+	+	+	+	+	+	+	+	+	+	+	+	71

Crosses indicate successful transmissions.

In a similar experiment (Table 6) two lots of five groups of eight aphids were moved on a succession of eight test plants. Aphids giving long infection feeding caused infection on the first day of test feeding, but only one of the 16 hr. infection-feeding groups transmitted on the second day, although all did later. In this experiment the aphids remained infective for 8 days, some infecting all the eight test plants. The aphids given short infection feeding, even after their delay period had passed, infected fewer plants than the aphids given long infection feeding, probably because they contained less total virus.

In the previous experiment with *D. tatula* all aphids reared on infected plants infected the test plants in the first 4 hr. Somewhat different results were obtained when potato plants were used. One such experiment is presented in Table 7. The aphids were reared on infected Majestic plants and then in groups of fifteen were placed for different periods on a succession of healthy sprouted tubers of the variety Arran Consul. Nine out of the ten groups caused infection, but only four groups infected the first plant on which they were left for 4 hr. The other five groups caused infection at different times up to 127 hr. after leaving the source of infection.

Several conclusions can be drawn and speculations made from the above results. The delay period is very variable and depends on all the factors which will influence the amount of transmission. These are: length of infection feeding, the number of aphids used per test plant, the amount of virus in the infected plant and the susceptibility of the test plant to infection. No other attempts were made, except

TABLE 6. *Effect of long and short infection feeding on the transmission of leaf-roll virus in a series of eight* Datura tatula *plants at day intervals*

	The infectivity of five groups of eight aphids reared on infected *Datura stramonium*					Aphids survived each transfer	The infectivity of five groups of eight aphids fed for 16 hr. on infected *Datura stramonium*					Aphids survived each transfer
Days	1	2	3	4	5		1	2	3	4	5	
1	+	+	+	+	+	40	−	−	−	−	−	40
2	+	+	+	+	+	39	−	+	−	−	−	36
3	+	+	+	+	+	37	+	−	−	−	+	34
4	+	+	+	+	+	35	+	+	+	−	+	31
5	+	+	+	−	+	29	+	−	+	+	+	31
6	+	+	+	−	+	27	+	+	−	−	+	27
7	+	+	+	−	+	22	+	−	−	+	+	19
8	+	+	+	−	−	19	+	+	−	−	−	18

Crosses indicate successful transmissions.

TABLE 7. *Transmission of potato leaf-roll virus to a succession of healthy Arran Consul potatoes by groups of fifteen aphids, reared on infected Majestic plants*

Feeding time on each test plant in hours ...	4	22	4	22	3	24	24	24	48
Group 1	−	−	−	−	−	+	−	−	−
2	+	+	−	+	−	−	−	+	−
3	−	−	−	−	−	−	−	+	+
4	−	−	+	−	−	+	−	+	−
5	−	−	−	−	−	+	−	−	−
6	−	+	−	−	−	−	−	−	−
7	+	+	−	−	−	−	+	+	−
8	+	+	+	−	−	−	+	+	+
9	+	+	−	+	+	+	+	−	−
No. of aphids survived on each transfer	135	133	131	130	129	116	94	64	26

Crosses indicate successful transmissions.

in using *D. tatula*, to reduce the delay period. It is likely that by varying the infection-feeding time until an optimum is found or by using large numbers of aphids the delay period could be shortened. The effect of susceptibility to infection on the length of the delay period is shown when the results in Tables 5 and 6 are compared with those of Table 7. All the aphids which were reared on infected plants produced infection in the first 4 hr. when *D. tatula* was used but only a few did so when potato was the test plant.

STRAINS OF LEAF-ROLL VIRUS

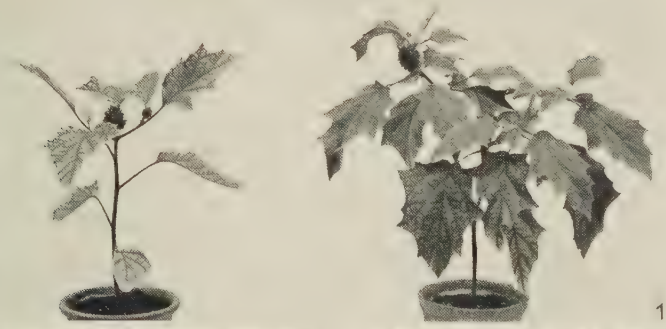
There is no record that potato leaf-roll virus exists in strains, though it is well known that plants which contract the disease in the field show symptoms of varying severity. An attempt was therefore made to elucidate this point. Tubers were saved from fifteen Majestic plants grown in different parts of the country which showed leaf rolling of various grades of severity but not obvious symptoms of mosaic. Next year the tubers were planted in the glasshouse and showed the same differences in severity of symptoms that were recorded the previous year. Inoculation to tobacco and *D. tatula* showed that they all contained potato virus *X*. The leaf-roll virus from these plants was transmitted by aphids to virus-free clones of Majestic and Craig's Defiance. Grafts were also made to U.S.D.A. 41956 which is immune from potato virus *X*. The progenies from these transmissions were planted for the next 2 years in the glasshouse and out of doors, and compared with the progenies from the original plants containing both viruses. Each year tests for the presence of potato virus *X* were made serologically and by inoculation to *D. tatula*.

It was concluded from these observations that the main reason for the striking differences in severity of symptoms in the original plants was the presence of potato virus *X* in strains of varying severity. After the fifteen isolates of leaf-roll virus were freed from *X*, infected plants differed little in appearance. It was, however, still possible to see variations in the amount of leaf rolling, general chlorosis and stunting. These differences remained constant in 2 successive years of observation. One isolate was particularly mild in its effects on all three varieties, both when grown in the glasshouse and in the field (Pl. 9, figs. 2–4). Craig's Defiance reacted more severely than Majestic to all the isolates.

The differences between isolates in the field were greater in 1949 than in 1950. This was attributed to soil and climatic conditions. In 1950 the plants were planted in better soil and higher rainfall favoured growth.

Microscopical examination of the stems of Majestic plants grown in 1949 in the field, using the phloroglucinol method, showed that the severity of phloem necrosis corresponded with the severity of leaf symptoms. The mild strain was distinguishable as producing very little phloem necrosis. When four of the isolates which were distinguishable in potato plants were transferred by aphids to *D. tatula* no differences in either chlorosis or stunting could be seen.

I wish to thank Miss Jean Matthews for her assistance in some of the experiments.



KASSANIS—*Transmission of leaf-roll virus by aphids*

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EXPLANATION OF PLATE 9

(Photographs by Mr V. Stansfield, F.R.P.S.)

Fig. 1. *Datura tatula* plants 2 months after infection with potato leaf-roll virus. Plant on the right received ammonium sulphate soon after the appearance of the symptoms.

Figs. 2 and 3. Two Majestic plants grown in the field infected respectively with a mild and a severe strain of leaf-roll virus.

Fig. 4. Two Majestic plants grown in the glasshouse infected respectively with a mild and a severe strain of leaf-roll virus.

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THE BIOLOGY OF THE RICE WEEVIL, *CALANDRA ORYZAE* (L.)

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(With 1 Text-figure)

The length of the developmental instars of *Calandra oryzae* has been estimated at relative humidities of 50, 60, 70 and 80 % at 21° C., at 70 % at 18, 25 and 30° C., and at 80 % at 18° C., by the dissection daily of samples of infested wheat-grains. The results are expressed as a median obtained by the method of probit analysis. Comparison of this method, with estimates of median and mean obtained by orthodox arithmetical methods from similar work on *Rhizopertha*, show that the probit method gives good estimates.

About 90 % of the eggs laid are fertile. Normally only one adult will develop in a grain, all other individuals being destroyed by cannibalism. The sex ratio is unity. It was not possible to cross *C. oryzae* and *C. granaria*.

The daily oviposition rate of *C. oryzae* at 17, 21 and 25° C. increases with relative humidity. There is a critical point at about 60 % R.H. below which egg laying declines rapidly, and mortality is high. At 100 % R.H. the oviposition rates per female per day are approximately 1·3, 2·5 and 3·4 at 17, 21 and 25° C. respectively.

In experimental conditions most eggs per grain are obtained by giving isolated females one grain each, but more eggs are laid by females given more than one grain. Daily egg output is reduced by grouping females or including males. In culture, depths of grain up to 7 cm. do not discourage egg laying.

Introduction

Grain weevils are probably the most destructive insect pest of stored cereals throughout the world, and consequently they have been the subject of a considerable amount of work. Nevertheless, there are many gaps in the knowledge of their biology, especially at the storage temperatures usual in Britain. This paper describes a miscellaneous group of experiments undertaken to fill some of the gaps.

The species used was usually the large strain of *Calandra oryzae* (L.) (Richards, 1944), since at the time Prof. O. W. Richards and his colleagues were engaged on a study of *C. granaria* (L.).

The methods used were similar to those described by Richards (1947). Most of the experiments were performed in dark constant-temperature rooms or incubators. The grain used was English wheat conditioned to a moisture content in equilibrium with the experimental humidity, viz.: 11·1 % for 50 % R.H.; 12·6 % for 60 % R.H.; 14·2 % for 70 %; and 15·8 % for 80, 90 and 100 % R.H. Most experiments were made in desiccators in which the humidity was controlled by solutions; a saturated solution of common salt for 73 % R.H., tap water for '100 %' and solutions of

caustic potash of known specific gravity for other humidities as follows: for 90 %, 1·115; for 80 %, 1·175; for 70 %, 1·230; for 60 %, 1·280; and for 50 %, 1·335.

Insects for experiment were bred in 2 lb. jam-jars containing 400 g. of wheat (about 10 cm. deep) and 200 weevils. After 1 month cultures were cleared of adults every week, or more frequently, to give groups of adults of known age. The adults were normally used alone or in small groups in small vessels. A number of kinds of vessel were compared, particularly glass specimen tubes closed by muslin covers, glass-topped pillboxes and holes bored in wooden blocks and closed with corks. No difference in oviposition performance was noted and generally 2 × 1 in. glass tubes were used thereafter.

Grains were dissected to show the presence of eggs, larvae, etc., after soaking for 24 hr. in 50 % alcohol. Alcohol killed the weevils in a few hours and the water softened the grain sufficiently to make it easy to find all the eggs and larvae present.

Rate of development and mortality of the immature stages

Although a great deal of work has been done on the duration of the egg, larval and pupal periods of *Calandra*, at the time the work described below was done, there was no information available on the duration of the separate larval instars of the grain weevils under constant conditions.

Since then, however, for *C. granaria*, Howe & Oxley (1944) have given results for 25° C., 70 % R.H.; Richards (1947) for 21° C. at 50, 60, 70 and 80 % R.H.; and Eastham & Segrove (1947) a very full set of results for six temperatures (15–30° C.) and five relative humidities (40–80 %). There is still no such complete information for *C. oryzae*.

Techniques

The eggs of weevils are laid in cereal grains, the whole developmental life being spent inside the one grain. The larva leaves the grain only in exceptional conditions which sometimes occur in culture. When this happens the larva does not enter another grain but it can subsist on flour, frass or grain detritus. Unlike *Rhizopertha*, it cannot complete development in flour, for not only is grain or macaroni required as a stimulus for oviposition, but the first-stage larva needs a firm medium to be able to feed.

In these circumstances it is not possible to follow individual insects through from egg to adult as with free-living stored-product pests. Even dissecting young larvae from grain and then growing them on flour would be unsatisfactory, for Howe (1950) has shown that *Rhizopertha* develops more slowly in wholemeal flour than in wheat.

The life cycle of weevils under any set of conditions must therefore be examined by taking regular samples of grains containing eggs laid over a known short period. As a rule only one weevil completes development in a grain, the rest being eliminated by cannibalism as egg, larva or pupa. This competition within a grain slows the development of the survivor (Birch, 1945), so it is desirable to use grains containing one insect only. Unfortunately, this is only possible in practice if so many grains

are provided for oviposition that many contain no insects, thus causing a great deal of fruitless dissection of grains. In this work, the grains used contained on average just over two insects per grain.

There were slight variations in the methods used to obtain infested grain for use at the different conditions investigated. At 18° C., fifty females were isolated, each in a $2 \times \frac{1}{2}$ in. glass tube with a single wheat grain and allowed to lay over 20 successive days at the appropriate humidity, each being given a fresh grain daily. The fifty grains obtained daily were shared between five $2 \times \frac{1}{2}$ in. glass tubes, the grains containing insects of appropriate age being dissected later, covering a period up to 100 days. A similar technique was used at 25 and 30° C. At 21° C. the experiment was set up at the same time and in the same way as described by Richards (1947). Thus the eggs for all four humidities were laid by the same 840 isolated females in 24 hr. periods at 73 % R.H., all laying periods following in quick succession. The 840 grains for each humidity were shared equally between five dishes, two being taken from each dish each day until finished. Emerged adults were removed as seen causing as little disturbance as possible.

The moisture content of the grain was adjusted before oviposition so that by the end of oviposition periods at 73 % R.H. it was at the moisture content appropriate to the experimental humidity. The solutions of caustic potash were renewed every 6 weeks, but there was some evidence that this was not often enough for the experiments at 21° C., in which the humidity may have risen as much as 5 % for a time. Ten grains were sampled and dissected daily.

Length of instars

The results obtained from this type of experiment consist of successive daily estimates of the proportion of the population in each instar. Richards (1947) recorded the day of the first appearance of each instar (as in Table 1), the day of last appearance and estimated, by inspection, an average instar length. He ignored the occasional laggard which, presumably due to some grain anomaly, occurs in these experiments. This is also done in this paper and is assumed to have been done by others working on weevils. Howe & Oxley (1944) present their results as

Table 1. *Day after egg-laying date of first appearance of instars of* Calandra oryzae *in English wheat*

Temp. (° C.)	R.H. (%)	Larva I	Larva II	Larva III	Larva IV	Pupa	Adult	Emergence
18	80	12	25	33	46	65	79	93
18	70	13	30	39	46	74	91	96
21	80	6	12	16	23	31	37	42
21	70	6	13	17	24	34	42	43
21	60	6	14	19	27	36	48	52
21	50	6	13	19	35	48	57	59
25	70	5	9	13	16	24	28	32
30	70	4	7	10	13	20	24	—

TABLE 2. *Median periods in days from oviposition to the end of the various development instars in* Calandra oryzae

Conditions			Larva					
Temp. (° C.)	R.H. (%)	Egg	I	II	III	IV	Pupa	Adult in grain
18	70	13·4	31·2	40·2	55·3	86·2	98·2	110·1*
	80	12·8	28·5	38·8	48·5	71·9	88·9	112·0*
21	50	6·3	17·3	32·7	43·9	57·7	64·0	73·7
	60	6·7	14·4	22·8	32·3	48·1	54·3	62·5
	70	6·9	15·0	19·6	27·6	38·7	46·9	54·0
	80	6·8	13·0	19·2	25·4	34·1	41·9	47·8
25	70	6·1	10·9	15·0	19·4	27·7	34·6	40·3*
30	70	4·5	8·6	11·8	15·5	23·9	28·2*	—

* Extrapolated.

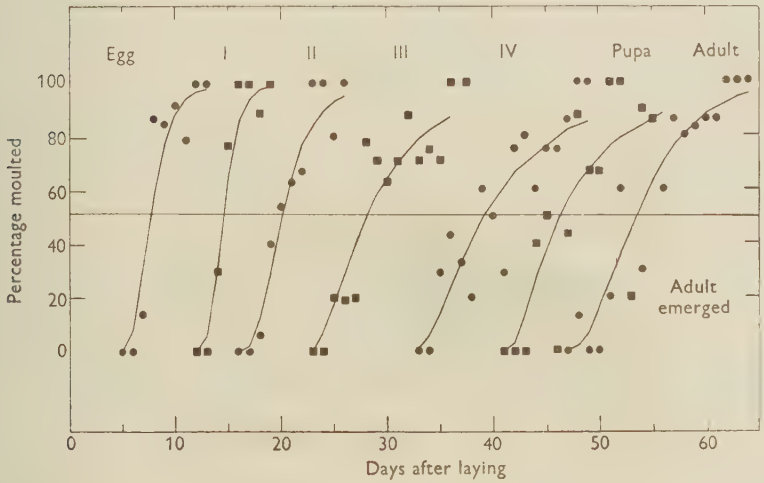

Fig. 1. Curves showing rate of change from one instar to the next, viz. egg to larva I, and thence II, III, IV, pupa, adult and adult emerged from grain, at 21° C., 70 % R.H. The curves were obtained by converting probit lines back to percentages. Black circles represent successively the actual percentage of stages reaching larva I and III, pupae and emerged adults in a sample, and squares larvae II and IV and adults inside grain. The 50 % line or median is shown.

histograms representing the daily proportion of each instar and give no numerical results. Eastham & Segrove (1947) plot points representing the proportion of each instar present on each day and draw an idealized curve through the points. They assume that the points where the curve for each instar cuts the neighbouring curves mark the average limits of the instar and they measure the time between these intersections to the nearest half day.

In general, these methods are probably adequate, but the work of Howe (1950) on *Rhizopertha* shows that this last method can give obviously inaccurate results where there is considerable variation between individuals. His instar curves overlap considerably and the mean time of change from one instar to the next was fixed by dividing the area of overlap equally by a line vertical to the time axis.

In this paper a statistic is found for instar length by using the probit method used in biological assay (Finney, 1947). The percentage moulted (or change from one instar to the next) is considered analogous to percentage kill and the age of the insect in days as analogous to the dose applied. The cumulative curve of moulting against time for each instar is very skew, but is normalized by substituting for time, the logarithm of time minus a constant. This use of probit analysis gives a median period for the completion of development of each stage up to the end of the instar concerned (Table 2), and a series of probit lines or curves representing the rate of change from one instar to the next (Fig. 1). In some ways a median is more satisfactory than a mean as a statistic for development periods, because it is less affected by the occasional abnormal individual. The labour of computation, however, is considerable for the information obtained.

Table 3 compares the results obtained by this method with those given by straightforward calculation of the mean using the results of Howe (1950) for *Rhizopertha* bred in flour. The estimates of the median both from the whole population and from daily random samples of ten individuals are close to the values calculated by simple arithmetic.

Table 3. *Information for* Rhizopertha *bred in flour at* 28° C., 70 % R.H., *from Howe* (1950)

(Mean and median in days found by normal arithmetic methods and the median also by the probit method using as a daily sample both the whole population (strictly a 'time mortality' curve) and a random sample of ten. The periods are to the end of each instar from egg laying.)

		Median		
Stage	Mean	Arithmetic	Probit	Sample of ten
Egg	7·12	7·05	7·17	7·16
Larva I	15·99	15·71	15·59	16·12
Larva II	21·65	21·56	21·30	21·04
Larva III	27·18	26·86	26·78	26·27
Larva IV	34·65	34·10	33·76	33·55
Pupa	40·09	39·93	39·21	39·82

Mortality and cannibalism

Under the conditions of these experiments, there is an average of more than one egg laid per grain. Since generally, only one adult weevil develops, some cannibalism is certain. On one occasion (at 21° C., 80 % R.H.) three live pupae were found in a single grain, three times two pupae were found and twice two adults which had certainly bred in the single grain.

It is very difficult to separate natural mortality from cannibalism, but it is possible to obtain some information at 21 and 18° C. Many infertile eggs look normal, so a time must be fixed before which eggs are assumed alive and after which are assumed dead. Thus at 21° C., the egg period is normally 6–7 days, so all eggs found from the eleventh day onwards are assumed dead. Cannibalism begins immediately after hatching and traces of killed individuals remain, but at 21° C., total mortality does not increase significantly until the twenty-fifth day. Egg fertility, estimated by counting the larvae and sterile eggs found between days 11–25 inclusive, was over 90 %. A confirmatory experiment done at 21° C., 60 % R.H., gave 89 % hatch. Fertility at 18° C., estimated from the samples examined for days 16–45 inclusive, was also about 90 %.

Total larval mortality in these early periods, estimated by counting living and dead larvae, varied between 15 and 45 %. An estimate of natural mortality of larvae and pupae at 21° C. was made by counting the dead individuals of each instar found in grains with no other occupant. The total number of grains containing only one insect was estimated by simple proportion from the number obtained in samples during the first 25 days. Such counts showed that natural mortality is slight except in the first larval instar. In this stage it is only 3·5 % at 80 % R.H. but rises to 30 % at 50 % R.H.

The further mortality caused by cannibalism due to overcrowding raises the total mortality in these experiments to 60–70 %. At both 18 and 21° C. the mean number of insects, live and dead, per attacked grain fell to 1·1–1·2, regardless of the original egg number (Table 4). Except at 50 % R.H. the fall in numbers is sudden, taking only about 10 days at the period of predominance of fourth-stage larvae. At 21° C., 50 % R.H., the fall is slow, taking from day 25 to day 60. Dead stages account for from 0·15 to 0·45 per grain attacked so that 0·8 to 0·95 live weevils emerge per grain attacked.

TABLE 4. *Estimates of eggs laid, mortality, and weevils emerging at* 21 *and* 18° C.

Temp. ...	21° C.			18° C.	
R.H. ...	80 %	70 %	50 %	80 %	70 %
Grains attacked (%)	81·7	71·7	74·3	59·2	57·8
No. of eggs laid	1680	1312	1400	947	983
Mean no. of eggs/attacked grain	2·5	2·1	2·2	1·6	1·7
Egg mortality (%)	5·3	11·9	5·4	10·3	8·3
Total mortality (%):					
By day 15	12·9	22·2	18·2	—	—
By day 20	15·5	42·0	29·4	—	—
By day 30	43·4	54·3	40·1	—	—
By day 50	—	—	—	17·3	19·3
Final mortality (%)	60·7	58·4	67·0	42·5	44·2
Insects/attacked grain:					
Final total	1·1	1·1	1·2	1·1	1·2
Final dead	0·2	0·2	0·4	0·2	0·25
Adults expected	660	550	464	545	549

Sex ratio

The number of adults obtained was small because a large proportion of the grains were removed before the adult stage was reached. The sex ratio of the adults obtained did not differ significantly from unity at any condition.

Adults obtained from other experiments and some of those taken from culture for experimental use were sexed, giving the ratio ♀ 3845 : ♂ 3877.

Oviposition experiments

(*a*) *Attempts to cross the species*

Reuter (1937) observed copulation between male *Calandra granaria* and female *C. oryzae* but not the reverse cross. After copulation viable eggs were produced but Reuter did not know if the females were virgin before the observed pairing. Therefore, in this work, isolated pairs of male *C. granaria* and virgin female *C. oryzae* were placed in $2 \times \frac{1}{2}$ in. glass tubes with English wheat of 15·9 % moisture content at 25° C., 80 % R.H. After a preliminary preoviposition period of 1 week with six grains, each pair was given two grains for successive periods of 48 hr.

Male *C. granaria* were provided by Mr A. F. O'Farrell, of the Imperial College of Science and Technology, who sexed them as described by Richards (1947, p. 5). They were kept for 6 weeks at 21° C., 80 % R.H., before use. To obtain virgin females of *C. oryzae*, cultures were set up by placing adults on grain of 15 % moisture content for 1 day at 25° C., 70 % R.H. One month later about fifty grains were removed and put singly in glass tubes. Each grain was inspected daily and, when an adult emerged, its sex was determined by examining the dorsal surface of the rostrum which is smooth and shiny in the female and coarsely punctured in the male. Some grains produced two adults of different sexes which were used as control pairs.

Thirteen experimental pairs were tested for seventy-six oviposition periods without producing an egg, no copulation being observed while grains were being changed. Ten control pairs of *C. oryzae* laid eighty-five eggs in fifty-five oviposition periods. These included three sterile pairs. Some of these pairs were seen to copulate. The male *C. granaria* were fertile with virgins of their own species. Thus it seems that crossing between these species is impossible. Virgin *C. oryzae* do not lay.

(*b*) *The influence of relative humidity upon egg production*

Three types of experiment were carried out to determine the effect of relative humidity upon oviposition (cf. Richards, 1947, p. 39).

In one, groups of females were moved from one humidity to another so that all groups experienced each humidity once during the experiment. In another, all the weevils used were tested under standard conditions and divided into groups of equal performance, each group then being used at an experimental humidity. In

the last, pairs of humidities were compared directly, two experimental groups of weevils usually being put at each humidity in turn.

The first kind of experiment was performed at 25° C. with ninety female weevils. Relative humidities of 50, 60, 70, 80, 90 and 100 % were maintained in desiccators, and a single grain of English wheat of appropriate moisture content was given to each female which was isolated in a 3 × 1 in. glass tube for a period of 48 hr.

Results are given in Table 5. After the experimental period (col. i), the surviving weevils were kept at the final relative humidity for three extra periods (col. iii), and then all were placed at 100 % R.H. for a final period (col. iv). It was obvious that the humidity experienced in the period immediately preceding the experimental humidity affected the number of eggs laid (col. iii). Oviposition was poor at humidities below 70 % R.H. (cols. i and iii) and the depressing effect persisted into the following oviposition period. In this experiment most of the periods at 50 % R.H. were followed by periods at 100 % R.H., causing a low egg number at this humidity. The period of 8 days at low humidities 50 and 60 % R.H. experienced by two groups markedly reduced their egg number at 100 % R.H. (col. iv). Mortality was high at 50 and 60 % R.H.

TABLE 5. *Oviposition by ninety female* Calandra oryzae *in single wheat grains at 25° C. in periods of 48 hr.*

(See text for explanation.)

Experimental		(i) Total oviposition by 90 ♀♀ at experimental R.H.	(ii) Total oviposition by 90 ♀♀ in period following experimental R.H.	(iii) Eggs per ♀ period at final R.H./eggs per ♀ period all R.H.	(iv) Eggs per ♀ period at 100 % R.H./eggs per ♀ period all R.H.
R.H. (%)	Moisture content (%)	($T \pm$ S.E.)	($T \pm$ S.E.)	(%)	(%)
100	15·8	250 ± 18·9	248 ± 18·9	151	169
90	15·8	323 ± 21·2	245 ± 17·0	148	165
80	15·8	274 ± 21·8	226 ± 16·0	142	230
70	14·2	263 ± 17·4	144 ± 17·8	112	187
60	12·6	170 ± 15·5	109 ± 14·3	66	126
50	11·1	94 ± 11·0	187 ± 17·2	48	105

Similar results were obtained in another experiment (Table 6) at 25° C. using twelve groups of seven isolated female weevils. Two groups were started at each humidity, six lots going to successively higher humidities and six going down the humidity gradient. Surviving weevils were kept four periods in the final humidity.

The second kind of experiment was carried out at 17° C. (Table 7A), 21° C. (B) and 25° C. (C, D). In A and C the original egg-laying tests were performed at 80 % R.H. and in B and D at 70 % R.H., each for four periods of 48 hr. The tested females in each experiment were divided equally into six groups, each with approximately equal egg output over both the total test period and in the last period of the test, and all with a similar proportion of good and poor layers. Thus in A each group laid on average about thirty eggs per period, but within a group the eggs

TABLE 6. *Mean eggs laid per period per female* Calandra oryzae *at various humidities at* 25° C.

Experimental R.H. (%)	Mean egg no. ±S.E.		Mean egg no. at final R.H. as percentage of egg no. of same groups at all R.H.	
	Up R.H. gradient	Down R.H. gradient	Up R.H. gradient	Down R.H. gradient
100	5·6±0·24	2·5±0·33	130	130
90	4·3±0·45	3·8±0·40	174	183
80	3·3±0·46	3·0±0·37	122	152
70	2·7±0·35	2·7±0·40	128	113
60	1·4±0·29	1·4±0·21	31	8
50	1·7±0·24	0·7±0·17	3	4

TABLE 7. *Oviposition of* Calandra oryzae *during periods of* 48 *hr. on single grains of wheat at various humidities*

R.H. (%)	Eggs/period during 4 test periods		Eggs/period during 6 experimental periods		Eggs/period on return to original R.H.		No. alive at end of experiment
	Per group	Per ♀±S.E.	Per group	Per ♀±S.E.	Per group	Per ♀±S.E.	
			A. 17° C., 15 ♀♀ per group				
100	29·5	2·0±0·18	43·0	2·8±0·22	37·2	2·6±0·19	14
90	29·8	2·0±0·15	38·8	2·7±0·19	32·5	2·4±0·19	14
80	29·8	2·0±0·17	29·5	2·0±0·19	23·5	2·2±0·20	10
70	29·5	2·0±0·17	20·5	1·4±0·14	35·2	2·5±0·21	14
60	30·3	2·0±0·17	7·0	0·7±0·19	8·8	1·7±0·47	4
50	30·0	2·0±0·18	1·3	0·1±0·12	1·7	1·3±0·62	1
			B. 21° C., 10 ♀♀ per group				
100	36·3	3·6±0·36	58·6	5·9±0·36	—	—	10
90	37·5	3·8±0·36	49·0	4·9±0·34	—	—	9
80	37·0	3·7±0·44	44·2	4·4±0·32	—	—	10
70	37·3	3·7±0·38	27·4	2·9±0·32	—	—	8
60	38·3	3·8±0·40	9·6	1·0±0·21	—	—	5
50	37·8	3·8±0·34	11·6	1·2±0·21	—	—	7
			C. 25° C., 12 ♀♀ per group				
100	88·8	7·4±0·52	104·5	8·7±0·44	86·5	7·2±0·50	12
90	84·5*	7·7±0·50	94·8	8·6±0·68	83·5	7·6±0·63	11
80	89·3	7·4±0·47	94·8	7·9±0·39	109·0	9·1±0·48	12
70	88·8	7·4±0·55	102·7	8·6±0·35	101·8	8·5±0·54	12
60	90·3	7·5±0·50	23·8	2·7±0·38	48·5	8·1±0·58	6
50	89·8	7·5±0·56	8·5	1·1±0·27	0·0	—	0
			D. 25° C., 12 ♀♀ per group				
100	46·8	3·9±0·37	107·5	9·0±0·68	69·0	6·3±0·94	11
90	45·5*	4·1±0·38	115·0	10·5±0·63	74·5	6·8±0·81	11
80	47·3	3·9±0·37	90·5	7·5±0·47	71·5	6·0±0·46	12
70	42·0*	4·2±0·42	52·3	5·2±0·60	39·5	4·4±0·67	9
60	47·8	4·0±0·38	43·3	3·6±0·45	47·0	4·7±0·87	9
50	47·0	3·9±0·41	4·5	0·4±0·17	3·0	3·0±3·0	1

* One or two escaped at start of experimental period and records expunged from test periods.

laid by a single female during the whole test period varied between 1 and 18. In each experiment, one group was placed at each relative humidity for six successive laying periods of 48 hr. In A, C and D the surviving weevils were returned to the original test humidity for four further periods.

These experiments show high egg output at high humidity with a sharp decrease at 60 % R.H. and below (Table 7). At these low humidities mortality was very high and the oviposition rate of the living weevils fell off progressively during the experiments. The oviposition rate of weevils not subjected to low humidity improved during the experiment, presumably due to the insects becoming adjusted to handling, and higher rates were obtained in the return periods than in the original test periods.

One or two additional direct comparisons of humidity were made. Thus, at 21° C., 100 and 50 % R.H. were compared with twenty fertilized females, keeping ten continuously at each humidity for 4 days. Each female was isolated in a 2 × 1 in. glass tube with a wheat grain of appropriate moisture content, this being renewed daily. At 50 % R.H., eggs were laid in only one grain compared with thirty-one out of the forty at 100 %. The daily egg rates were 0·03 and 1·48 per female respectively.

Comparisons of 100 and 90 % R.H. (at 25° C.) and 100 and 80 % R.H. (at 21° C.) were made in a similar way except that in each experiment the two groups were subjected alternately to each humidity. In both, laying was better at 100 % R.H. At 21° C., when twelve groups of four female weevils were given four grains a day for 12 days, 83 % of the grains contained eggs at 100 % R.H. compared with 64 % at 80 % R.H., the mean daily egg numbers per female being 1·35 and 0·96 respectively. At 25° C., sixty-one single females were given single grains for periods of 48 hr. and the egg numbers per female per period were 5·5 at 100 % R.H. and 5·1 at 90 % R.H. This batch was divided into two which were kept continuously at one or other humidity. The individuals kept at 100 % R.H. increased their egg number per female per day from 5·4 to 6·8, while that at 90 % R.H. changed from 5·8 to 5·4.

The effect of humidity on egg hatching and on the proportion of grain used for oviposition is given in Table 4. In an additional experiment at 21° C. the percentages of eggs failing to hatch at 50, 60, 70 and 80 % R.H. were 11·0, 10·9, 10·0 and 6·0 respectively.

Humidity during development has some effect on adults. Weevils bred at low humidities die more quickly, and a larger proportion of the females are infertile compared with weevils bred at higher humidities. The fertile females bred at low humidities are very good layers.

(*c*) *The influence of temperatures on egg production*

The oviposition rates of groups of isolated females were measured at 100 % R.H. at 17, 21 and 25° C. The insects were bred at the experimental temperatures, the adult age of those bred and tested at 17, 21 and 25° C. being 90, 60 and 30 days

respectively. After the original oviposition test each experimental group was divided into three similar subgroups of equal performance, and these were placed one at each of the three experimental temperatures for a further test. Then the weevils were returned to their original temperature conditions and another measurement of oviposition rate was made. The entire experiment was repeated and all results are given in Table 8.

Table 8. *Oviposition rate in periods of* 48 *hr. by isolated* Calandra oryzae *females in a single wheat grain at* 100 % R.H. *at various temperatures*

No. ♀♀ per subgroup	Breeding temperature (° C.)	Experimental temperature (° C.)	Four test periods (M. ± S.E.)	Six experimental periods (M. ± S.E.)	Four periods at original temperature (M. ± S.E.)
5	17	17	2·3 ± 0·27	1·9 ± 0·22	2·1 ± 0·30
		21	2·2 ± 0·30	4·4 ± 0·43	2·0 ± 0·20
		25	2·3 ± 0·46	5·8 ± 0·74	2·0 ± 0·27
10	21	17	4·2 ± 0·20	3·4 ± 0·20	5·5 ± 0·40
		21	4·2 ± 0·40	4·9 ± 0·40	4·8 ± 0·56
		25	4·2 ± 0·42	9·3 ± 0·42	5·9 ± 0·46
9	25	17	4·7 ± 0·52	1·9 ± 0·19	5·0 ± 0·55
		21	4·6 ± 0·56	5·2 ± 0·39	7·1 ± 0·53
		25	4·6 ± 0·32	7·6 ± 0·61	8·0 ± 0·88
9	17	17	1·6 ± 0·27	1·8 ± 0·26	1·8 ± 0·37
		21	1·6 ± 0·24	4·9 ± 0·42	3·0 ± 0·30
		25	1·5 ± 0·25	6·3 ± 0·67	2·9 ± 0·40
10	21	17	4·5 ± 0·41	3·8 ± 0·26	5·4 ± 0·53
		21	4·4 ± 0·48	5·9 ± 0·34	5·4 ± 0·59
		25	3·8 ± 0·41	7·0 ± 0·61	4·4 ± 0·56
11	25	17	7·1 ± 0·48	4·3 ± 0·23	8·2 ± 0·70
		21	6·9 ± 0·49	6·1 ± 0·34	7·5 ± 0·58
		25	6·7 ± 0·53	9·5 ± 0·50	7·7 ± 0·45
	Oviposition temperature:				
		17	1·8 ± 0·12	3·0 ± 0·11	2·4 ± 0·15
		21	4·2 ± 0·17	5·3 ± 0·16	5·2 ± 0·21
		25	6·0 ± 0·23	7·3 ± 0·24	7·3 ± 0·26

M. = mean per ♀ per period.

Again an improvement in oviposition during the experiment is apparent. The daily oviposition rates per female over both experiments average 1·3 at 17° C., 2·5 at 21° C. and 3·4 at 25° C., that is approximately in the ratio 3:6:8.

(*d*) *The influence of the number of grains given per female upon egg output*

The effects of providing two grains instead of one per female and those of using more than one female in each container were studied superficially at 21° C., 100 % R.H., using glass-topped pillboxes of diameter $1\frac{1}{4}$ in. More eggs per female were obtained by giving two grains (Table 9). Most eggs were laid per grain with one grain per female. Both these differences were statistically significant (*t* test). Fewer eggs per female were laid when two females were placed in one container and

still fewer with four in a container. The presence of males also reduced considerably the oviposition rate (Table 9). These results are essentially the same as reported by Maclagan & Dunn (1936), who worked over a wider density range with groups of 4 to 128 insects on 25 to 800 grains. Their conclusion that maximum egg laying is not attained unless many grains are available for oviposition is supported by the fact that the maximum rate of egg laying in this work with only one or two grains available per female (4·5 per day) is well below the figure they obtained with many grains per female (6·75 per day).

TABLE 9. *Daily oviposition rate and percentage of grains utilized for laying using small numbers of weevils and grains in a* $1\frac{1}{4}$ *in. pillbox at* 21° C., 100 % R.H.

(The figures in brackets are results obtained in three periods following the removal of males.)

No. of weevils/pillbox					
♀	♂	No. of grains/pillbox	Total grains dissected	Grains utilized (%)	Eggs/♀/day
4	0	4	100	82	1·8
4	0	8	88	70	2·6
2	0	4	192	77	2·7
4	0	4	64	73	1·4
2	0	4	64	70	2·0
4	0	4	48	83	1·9
1	0	1	42	81	2·1
1	0	2	90	78	3·1
1	1	1	40 (30)	35 (93)	0·4 (2·8)
1	1	2	80 (36)	33 (78)	0·8 (3·0)
1	0	1	40 (36)	78 (83)	1·5 (2·7)

(*e*) *The influence of depth of culture on egg production*

The deeper layers of ordinary cultures maintained in jam-jars tend to moisten and cake, and weevils avoid the mouldy grain. When caking was prevented, the depth of culture did not affect the rate of oviposition in an experiment lasting 15 weeks at 21° C., 70 % R.H. Cultures were made up with 200 g. of English wheat, moisture content 15·9 %, to a depth of 7 cm. in 1 lb. jam-jars, by adding 100 weevils of each sex. Every week the top half of the culture was carefully poured off and 100 grains sampled from each half. Dissection of these grains revealed no material difference between samples from top and bottom at any time during the experiment. In each the total living stages found per 100 grains rose to the region 110–140 by the third week and remained at that level; third- and fourth-instar larvae appeared by 6 weeks and settled down at 30–45 per hundred grains, the younger stage then falling to about 80–100 per 100 grains. After 4 weeks the percentage of grain containing life remained at about 70–90 %.

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THE ECOLOGY OF *ANISOPUS FENESTRALIS* SCOP. (DIPTERA) IN SEWAGE BACTERIA BEDS

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(With 2 Text-figures)

The assessment of populations of *Anisopus fenestralis* (Scopoli, 1763) in sewage bacteria beds by the trapping of adults at the surface has been previously proved to be unsatisfactory. Methods were therefore developed for determining the numbers of egg masses, larvae and pupae as well as the total organic matter in a unit volume of bed. The relative larval populations of three areas under investigation were related to the loading of the beds as measured by the sewage strength and rate of application. In the upper 2 ft. 6 in. the numbers of larvae and pupae per unit volume of bed decreased with depth. There was no evidence of any vertical migration of the larvae before pupation. The horizontal distribution of larvae was found to be affected by the method of distribution. In beds served with fixed spray jets larvae are more abundant nearer the jets than at some distance away. In beds served with travelling distributors the larvae are more abundant in the zones below the jets than in the drier intermediate zones. In both cases relatively higher percentages of pupae in the drier zones might indicate a horizontal migration of larvae before pupating or of the pupae themselves.

The incidence of the larvae throughout 1949 in all three beds showed recurring peaks. In the following year a more intensive investigation on one area showed that this was due to successive generations of the fly; the proximity of the peaks is determined mostly by temperature and their size by the amount of food available in the bed during the larval grazing phase.

In the area not treated with insecticide intraspecific competition during a period when food was limiting, due to depletion by the previous generation, resulted in a natural reduction in the population. In the area treated with insecticide the food was retained and this natural control was thereby delayed.

Introduction

Grazing organisms such as fly larvae, worms and Collembola in sewage bacteria beds play a major role in the removal of accumulated organic matter and bacterial and fungal film which would otherwise eventually choke the beds. In beds treating domestic sewage there is a mixed fauna of these grazing organisms; trade wastes, however, limit the fauna to one or few species (Reynoldson, 1948). At the Minworth Works of the Birmingham Tame and Rea District Drainage Board the fauna is thus limited; the dominant fly for most of the year being *Anisopus fenestralis* (Scopoli, 1763). Although beneficial in its larval phase the adult fly, because of its shade-seeking habits, has caused nuisances in the neighbouring villages. Methods of chemical control were investigated, and these have been described (Tomlinson, Grindley, Collett & Muirden, 1949; Jenkins, Baines & Hawkes, 1949). Estimation

of the *A. fenestralis* populations of bacteria beds by trapping the flies in trays on the surface of the beds was considered unsatisfactory because of the effects of climatic variations on the numbers trapped (Hawkes, 1951). To apply the control measures most effectively a knowledge of the incidence and distribution of the different stages in the bed was considered necessary. An ecological study was therefore carried out and the effects of a single application of insecticide were also investigated. The simplified habitat of bacteria beds, as previously discussed by Reynoldson (1948), provides an ideal field for the study of the relationship between an animal population and its food supply.

Description of Minworth bacteria beds

Only the final stages of purification are done at Minworth, the removal of suspended matter by sedimentation having been carried out at another works farther up the Tame valley. On arrival at Minworth a portion of the settled sewage is partially purified by activated sludge before being applied to some bacteria beds; the remainder of the sewage, after further sedimentation, is applied to other beds. Two methods of distribution of the sewage are in use. On some beds it is distributed from fixed spray nozzles projecting from pipes laid on the surface of the bed. These beds thus receive a continuous spray of sewage, but for several hours each day depending upon the flow of sewage, the beds are rested. On the surface of these beds there develops each winter a thick fungal growth of species of *Fusarium*, *Oospora* and *Sepedonium*. On other beds the sewage is applied through travelling distributors which provide an intermittent dose on to each part of the bed about every 15 min. On these beds no surface fungal growth develops, but the surface is coloured by a thin film of unicellular Chlorophyceae. For the purpose of the investigation three areas B, D and F were chosen which were operated under these different conditions, Area B was treating the partially purified sewage distributed through fixed spray nozzles, whereas area D treated only settled sewage similarly distributed. The third area, F, treated partially purified sewage distributed through travelling distributors. The filtering medium varies in size but for the most part is of 2–3 in. crushed stone in beds B and D and of round gravel of the same size in F bed.

General methods

Assessment of eggs, larvae and pupae. Canisters 6 in. deep and 6 in. diameter, the base and sides being perforated with seven and sixty $\frac{3}{4}$ in. holes respectively, were buried in the bed at the required levels after being filled with medium removed from the corresponding level. The positions and depths of the canisters were marked with metal flags. The canisters were left in the bed for at least 12 weeks, then removed according to a sequence previously determined at random.

The eggs, larvae and pupae were removed from the contents of each canister by washing in a trough on a grid of $\frac{1}{4}$ in. mesh. The flow of water carried the organisms through the grid and along the trough into a sieve with $\frac{1}{16}$ in. perforations which

strained out the eggs, pupae and larger larvae of *A. fenestralis*; the young larvae and other grazers such as *Achorutes subviaticus* Begn., and *Psychoda* spp. larvae, however, passed through. The organisms were then counted over a sectored illuminated counting chamber.

Assessment of adults. Adult flies were assessed by inverting metal tray traps, 1 ft. square and 3 in. deep over the bed for 24 hr. and then chloroforming the flies collected. As previously reported (Hawkes, 1951), this method does not give valid results throughout the year because of effects of climatic variations on the trapping method.

Determination of organic matter in bed. Using the same type of canister and method as described above, samples were removed from the bed. From these the film and accumulated organic matter were removed in a revolving drum-washing apparatus.

Determination of population size, distribution and seasonal incidence of Anisopus fenestralis *in three beds working under different conditions* (1949)

From each of the three areas, B, D and F, eighteen samples at ten locations were removed each month. Ten (1*a*–10*a*) were from 0 to 6 in. depth, five (2*b*, 4*b*, 6*b*, 8*b* and 10*b*) from 12 to 18 in. and three (2*c*, 6*c* and 10*c*) were from 24 to 30 in. To investigate the distribution of larvae and pupae at different distances from the spray jets the canisters on areas B and D were set at 1, 2, 3, 5 and 8 ft. from the jets. Two samples were removed monthly from each of these five positions at the surface, one sample from each position at 12 in. deep, and one sample from the 1, 3 and 8 ft. distances at 24 in. The numbers of larvae and pupae of *A. fenestralis* in each sample were determined. The means of the number of adults trapped daily in three trays on each area were calculated as weekly averages.

Comparison of populations and seasonal incidence in the three areas

The means of the numbers of larvae and pupae found in the eighteen samples removed monthly from each area are expressed graphically in Fig. 1, together with the monthly mean of the numbers of adults trapped. The results show throughout the year a succession of three or four peaks in the larval population. The size of the peaks varied from area to area and throughout the season within the same area. In area B there were three large peaks during the first half of the year followed by reduced numbers during the months of July to September, with another smaller peak in the autumn. In area D two very large peaks occurred in the first half of the year, again followed by reduced numbers in the period July to September but with a third large peak late in the year. There was only one large peak in area F; this occurred early in the year and was followed in May by a second peak of reduced size, and in September by a third reduced peak. A fourth, larger, peak occurred towards the end of the year.

A similar periodicity has been recorded in the populations of the sewage flies

Spaniotoma minima Mg. and *Psychoda alternata* Say. (Lloyd, 1941, 1943). The relative size of the peaks throughout the year could be accounted for by the varying amounts of food material available. Although this was not measured during 1949, the fungal film in B and D beds was clearly plentiful during the winter and spring but became scanty by the end of June and did not build up again until October. In bed F the film accumulation was at all times less than in the other beds but followed a similar cycle.

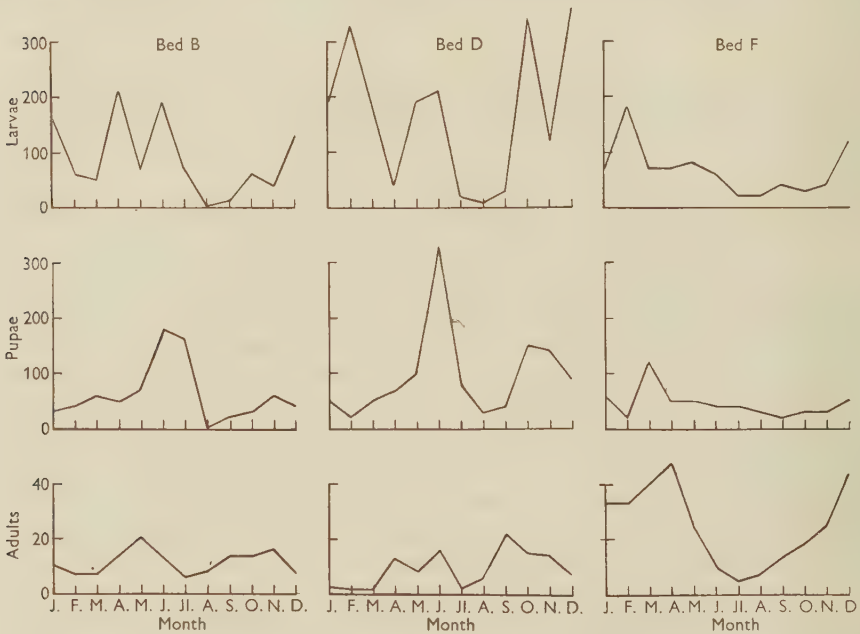

Fig. 1. Seasonal incidence and relative populations of *Anisopus fenestralis* in beds B, D and F.

Table 1 shows that bed D supported the largest larval population, B the next largest and F the least. Although no measurements of the organic matter in the beds were made, the sewage strength and volume treated per unit area of bed may be taken as a measure of the food available, and Table 1 shows that this factor can be correlated with the larval populations.

The pupal population as shown in the graphs does not follow the larval periodicity. This may be due to some peaks not being recorded because of the infrequency of sampling or to a natural high mortality rate of the larvae at some periods. In beds B and D there was a large pupal peak corresponding to the June larval peak and a smaller one in the autumn. In bed F a large pupal peak corresponded to the first generation of larvae. The cleaner conditions in this bed were probably more

favourable for pupation; these conditions were not present in the other beds until later in the year.

TABLE 1.* *Relationship between bed loading and abundance of larvae, pupae and adults of* Anisopus fenestralis

Bed	Bed loading (lbs. B.O.D.†/acre/day)	Mean no. of larvae	Mean no. of pupae	Mean no. of adults
B	1848	87	61	10
D	>2900, estimated max. 4850	169	95	9
F	1811	66	44	24

† The B.O.D. (Biochemical Oxygen Demand) is a measure of the oxidizable organic matter in the sewage.

The incidence of the adults as measured by tray trapping shows a summer reduction; the period of maximum abundance occurring earlier in bed F than in B or D, as would be expected from the incidence of the pupae. The comparative figures of the mean numbers of adults trapped on the three beds would suggest a higher mortality rate of the pupae in the dirtier beds. It may, however, be due to a direct effect of the thick growth on the trapping method.

Vertical distribution within the beds

Using the results obtained from five samples from the surface, five at the 12 in. level and the three at the 24 in. level each month, the vertical distribution in the upper 2 ft. 6 in. of the beds was investigated. For each month the means of the numbers at the three levels in each area were calculated. Because of the month-to-month variations in the totals, these means were expressed as percentages of the total. Table 2 shows the means of these monthly percentages for the whole year for both larvae and pupae.

TABLE 2. *Percentage vertical distribution of larvae and pupae in upper 2½ ft. of bed*

	Depth (in.)	Bed B	Bed D	Bed F
Larvae	0–6	50±5·6	52 ±3·76	40 ±3·4
	12–18	31±4·2	29·2±2·1	32 ±2·9
	24–30	19±3·9	18·8±0·86	27·5±3·1
Pupae	0–6	52±6·5	56 ±4·7	38 ±4·3
	12–18	29±4·55	26 ±2·8	38 ±4·5
	24–30	19±3·46	18 ±3·2	24 ±3·15

Throughout, the numbers of larvae and pupae decrease as depth increases. The differences were most significant in D and least in F. This vertical distribution can be correlated with the greater development of the fungal growth nearer the surface.

* The full biological data relating to this paper are lodged in the General Library of the British Museum (Natural History), Cromwell Road, London, S.W. 7.

This was especially apparent on beds B and D; on bed F, because of the different method of distributing the sewage, the film was more evenly distributed throughout the depth of the bed. There is no evidence of vertical migration of larvae before pupation, as occurred in the case of *Metriocnemus longitarsus* Goet. (Dyson & Lloyd, 1935), the proportional distribution of the pupae being much the same as that of the larvae.

Horizontal distribution within the beds at three levels

Although the fixed spray jets were designed to give even distribution of the sewage over the bed, varying sewage flows and other factors caused the areas nearer the jets to receive more sewage and consequently a greater accumulation of film than those at a distance. The results of the larval and pupal determinations at five positions at the surface and 12 in. levels, and the three at the 24 in. level were each expressed as percentages, and the means of the twelve monthly figures for each area are given in Table 3. These show that although there is no gradual zonation with distance two population zones can be distinguished. On bed B at all three levels the inner three positions have higher populations than the outer two. In bed D, it is only the inner two positions which have higher populations than the outer three, this not being so, however, for the lowest level where presumably the sewage had become more evenly distributed. Comparison of the proportional distribution of the pupae with that of the larvae indicates that there was some outward horizontal migration to the drier regions, the proportion of pupae at the 8 ft. distance being greater than that of the larvae at that distance.

Table 3. *Percentage horizontal distribution of larvae and pupae in relation to distributor jets*

Stage	Bed	Depth (in.)	Distance from jet (ft.) 1	2	3	5	8
Larvae	B	6	29±8·1	21±8·9	24·5± 4·3	12·5±4·4	13±4·2
		18	27±9·7	26±9·0	24 ± 6·1	8 ±2·4	14±3·7
		24	36±8·3	—	45 ±10·0	—	19±4·3
	D	6	22±5·9	28±5·3	15 ± 4·9	19 ±4·0	16±3·25
		18	29±6·5	31±5·4	14 ± 7·2	12 ±2·7	14±3·45
		24	49±8·6	—	19 ± 4·5	—	32±8·5
Pupae	B	6	32±6·6	26±8·25	12 ± 3·35	8 ±1·58	22±7·3
		18	26±7·15	21±6·7	20 ± 6·35	10 ±4·55	23±3·65
		24	38±7·9	—	40 ± 8·85	—	22±4·75
	D	6	21±5·3	28±6·95	15 ± 4·55	20 ±4·15	15±3·75
		18	24±5·7	20±3·2	19 ± 4·75	15 ±2·9	22±5·2
		24	37±8·95	—	27 ± 7·5	—	36±7·6

In bed F, the distribution of the sewage through travelling distributors produces a different type of zonation. Immediately below each travelling jet is a strip which receives every 13 min. a jet of sewage, while between these jets is a zone which is kept moist only by splashing and condensation. This zonation is indicated on the

surface by a striped appearance due to the growth in the 'subjet' position of unicellular algae, these being grazed away in the 'interjet' zone by *Achorutes*, which are unable to exist under the jet. Because of the close proximity of the jets this zonation was only evident near the surface. On other beds, however, with similar distribution mechanisms but with wider spaced jets a broader zonation was produced permitting representative sampling. On three occasions during the first half of the year, nineteen samples were taken from each of the two zones at three different levels.

TABLE 4. *Percentage horizontal distribution of larvae and pupae in the subjet and interjet zones of a bacteria bed served with a travelling distributor*

	Larvae		Pupae	
Depth	Subjet zone	Interjet zone	Subjet zone	Interjet zone
Surface	69	31	6	94
18 in.	87	13	20	80
24 in.	76	24	50	50

The relative numbers of larvae and pupae in the two zones at each level were calculated. Table 4 shows that there is a horizontal distribution of the larvae determined by the positions of the jets. Whereas the larvae prefer the 'subjet' position the pupae were most numerous in the drier 'interjet' zone, this being most marked near the surface and less at the 18 in. level; at the 24 in. level the distribution in the two zones was equal. These relative distributions of larvae and pupae indicate a migration to the drier zones either of the larvae prior to pupation or of the pupae themselves which are capable of some movement. Egg masses of *Anisopus fenestralis* and the hydrophilid beetle *Cercyon ustulatus* Preys. were found in the 'interjet' zone. *Lumbricillus* sp., however, was for the most part found in the 'subjet' zone.

Film accumulation and the incidence of eggs, larvae, pupae and adults of Anisopus fenestralis *in bed F* (1950) *and some effects of a single treatment of insecticide*

A bed in block F was divided into two areas; area 1, treated with insecticide 7 weeks after observations started, and area 2, not treated. From each area eight containers, previously sunk below the surface, were removed weekly, five of which were used for fauna determinations and three for film determinations. During two 24 hr. periods each week flies were taken in three tray traps on each of the two areas. The means of the two weekly trappings for each area were calculated, and the mean daily bed temperature. During the seventh week of the experiment benzene hexachloride (BHC) was applied to area 1 as a water dispersible powder at the rate of 1·3 lb. of the active γ-isomer per acre.

Graphs (Fig. 2) were plotted, using the weekly mean figures for each area. To smooth out weekly irregularities in the results caused by the method of sampling, a 3-week moving average was applied to the film accumulation figures.

Fig. 2 shows that in both areas there was a periodicity in egg-laying activity as shown by the recurring peaks throughout the year. Commencing with the egg peak in the sixth week and assuming a thermal constant for the development of *A. fenestralis* of 814 days degrees (Khalsa, 1948) the theoretical recurrences of this peak were calculated and indicated on the graphs by vertical lines *A* to *F*. These calculated

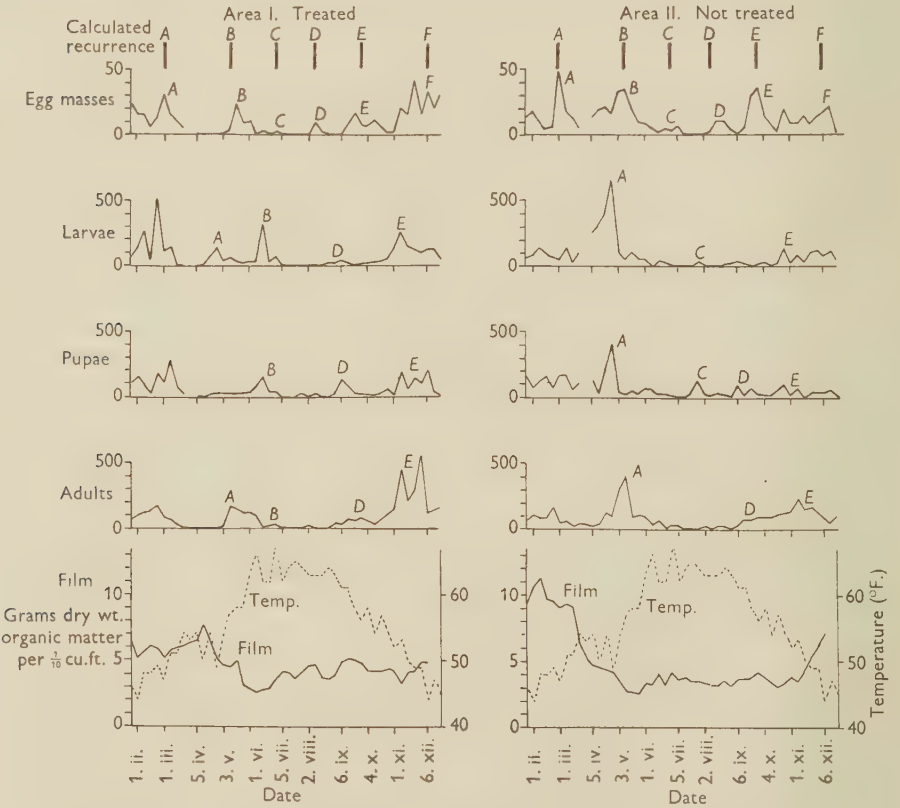

Fig. 2. Incidences of stages of *Anisopus fenestralis* in relation to film accumulation and temperature in bed treated with insecticide (BHC) and in bed not treated.

times of recurrence coincide with the peaks. Peak size varies considerably throughout the year, being smaller during the summer. Resultant larval, pupal and adult peaks are usually evident, the coincidence of the larval and pupal peaks being explained when it is recalled that only the larger larvae are retained in the wash. The variation in the size of the peaks throughout the year and on the two areas is related to the food supply in the beds. At the beginning of the experiment the film accumulation in the untreated area was the greater, but for a period coinciding

with the grazing of generation (*A*) of larvae there was a marked unloading of this film. Although a second generation of eggs (*B*) occurred this did not produce detectable peaks of larvae, pupae or adults. The third and fourth generations (*C* and *D*) were also only represented by small peaks. It would appear that although the (*A*) generation had ample food and successfully gave rise to adults which produced eggs, their grazing had so depleted the food supply that intraspecific competition between the second generation (*B*) of larvae resulted in a high mortality. This intraspecific competition when food is limited has been demonstrated in culture work (Hawkes & Jenkins, 1951). The small peaks throughout the summer can also be explained in terms of reduced food supply in the bed.

In area 1 the egg peak (*A*), because of subsequent treatment with insecticide, produced greatly reduced larval and adult peaks (*A*). The suppression of this larval generation by insecticide treatment resulted in decreased grazing, giving only slight reduction in the film at the period of maximum unloading in the untreated area (Fig. 2). However, the second generation of eggs (*B*) gave rise to more larvae and pupae than in area 2, the food supply in area 1 not having been depleted by the first generation. The grazing of this second generation resulted in further unloading of the film which limited the food available for the following generations. The larger size of the (*E*) generation of larvae in the treated area 1 than the corresponding generation in area 2 is related to the greater film accumulation in the former area. With a fall in temperature in the autumn there was a noticeable increase in film, this being subjected to a further unloading in area 1 because of the larger larval population.

Discussion

This investigation of the *A. fenestralis* populations in bacteria beds resolves itself into a study of the relationship between an organism and its food supply. The relative abundance, the seasonal incidence, and the vertical and horizontal distributions of the larvae within the bed are all determined by this factor. The population size, however, in turn affects the subsequent food supply and thus a dynamic state of balance is set up. A similar equilibrium between *Psychoda alternata* and the fungus *Oospora* occurred in the beds at Huddersfield (Reynoldson, 1948). Here there was a differential reaction of the fly and fungus to temperature changes. Because of the lower threshold of development of *Anisopus fenestralis*, 2·1° C. (Khalsa, 1948), it is able to complete its life cycle throughout the winter months. The shortening of the life cycle with increase in temperature suggests an increase in the grazing of the larvae and this, rather than an increased population, would seem to be responsible for the spring depression in the film accumulation, which in turn resulted in a depression of the larval population. The maintaining of the film at a low level throughout the summer months in the absence of a large larval population may have been due to the presence of other grazing organisms such as *Achorutes subviaticus* and *Lumbricillus lineatus* Mull., or to bacterial oxidation of the film at high temperatures (Heukelekian, 1945). With a decrease in temperature

in the autumn the film again accumulated and resulted in a recovery in the *Anisopus fenestralis* population. Tomlinson & Stride (1945) showed that the amount of film in beds operating on single filtration increased with increasing filter load. They also correlated this increase with an increase in the *Psychoda* population. In *Anisopus fenestralis*, however, they found no such relationship. The conflicting results may be accounted for by the different methods of assessment used. The previous workers used as their index the numbers of adults trapped on the surface of the bed. Table 1 shows that by using the number of adults trapped a different conclusion can be drawn, but it is considered that this does not give a valid estimate of the fly population of a bed. The previous workers reported that the emergence of flies from the filters into the traps may have been impeded by the clogging of the surface of the bed with film, and they therefore based their conclusions on figures obtained between May and October. It is probable that in beds having a mixed fauna which show interspecific competition no such simple relationship between the *A. fenestralis* population and filter loading exists.

Lloyd (1941, 1943) demonstrated the seasonal rhythm of the sewage flies *Psychoda alternata* and *Spaniotoma minima*, and considered that because of irregularities in temperature changes throughout the year populations of all rapidly breeding insects not having a diapause would in time assume such a periodic rhythm. The present work demonstrates that such a rhythm exists also for *Anisopus fenestralis* in the beds at Minworth. In *Psychoda alternata* and *Spaniotoma minima* there were two or more parallel generations. At summer temperatures, because of the speeding up of the relatively short life cycles, these peaks may run together. On the other hand, a sudden fall in temperature in the autumn may cause a peak to split. In *Anisopus fenestralis* only one succession of generations was traceable, but there was some indication of the splitting of peaks with the fall in temperature in the autumn.

Practical recommendations for the control of the fly can be based on the conclusions of this investigation. The fly population is checked by limiting its food supply, that is by reducing the amount of accumulated organic matter in the bed. At equivalent rates of application this may be achieved by a more efficient removal of the solid matter before applying the sewage to the beds. Methods of restricting the accumulation of film by recirculation and double alternate filtration would also result in a depression of the fly population. With the normal single filtration method, any method of distribution which inhibits the luxurious surface growth of fungus will also limit the fly population. In this respect distribution of the sewage by mechanical distributors would appear to be preferable to distribution through spray jets. A further measure would be the removal or neutralization of toxic components of the sewage to encourage other grazing organisms to compete with the larvae of *A. fenestralis* for the food available. All these methods of control by limiting the food supply do not affect the dynamic balance, this being established at a lower level. In contrast, artificial means of control by insecticides upsets this

balance, the grazing population being suppressed whilst the film accumulation remains at a high level. In practice the results of such a state are twofold. Unless other grazing organisms are able to establish themselves this unbalanced state can only be maintained by continuous and expensive treatment. Secondly, the film accumulation in the absence of the larvae may be sufficient to impair the efficiency of the bed.

Under present operating conditions, however, insecticides must be used. For their efficient application, it is necessary to have the information on larval distribution and seasonal incidence, provided by this investigation, and on factors affecting the seasonal egress of the fly from the beds (Hawkes, 1951). The addition of the insecticide in the sewage distributed to the bed would seem to be sound practice as the more vulnerable larval stage is distributed proportionately to the sewage applied. The effects on film accumulation and bed efficiency of complete suppression of the *A. fenestralis* population throughout the year are now being investigated. Until the results of these further investigations are available the grazing action of the fly larvae should be retained; insecticide treatment only being given when necessary to avert nuisance.

This investigation was carried out as part of the fly control programme of The Birmingham Tame and Rea District Drainage Board, and is published by permission of Mr F. C. Vokes, B.Sc., M.I.C.E., Engineer to the Board. I am grateful to Dr S. H. Jenkins for his guidance in the preparatory stages of the work and for his continued interest throughout. I am also indebted to Mr K. Snape for assistance in the routine counting and to the staff at Minworth Works.

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STUDIES IN THE MODE OF ACTION OF INSECTICIDES

I. INJECTION EXPERIMENTS ON THE ROLE OF CHOLINESTERASE INHIBITION

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Acetylcholine, choline chloride, acetyl-β-methylcholine, benzoylcholine, carbamyl choline, adrenaline and *d*-tubocurarine are non-toxic when injected into the locust. Prostigmine is also non-toxic, and eserine considerably less toxic to the locust than to man.

The toxic effect of tetraethyl pyrophosphate (TEPP) cannot be antagonized by injection of atropine or enhanced by *d*-tubocurarine.

The injection of acetylcholine chloride following injection of TEPP does not affect subsequent mortality.

These findings are discussed, and it is suggested that the physiology of the nervous system of the insect is unlike that of the mammal, neither cholinesters nor adrenaline being concerned in it. Phosphorus insecticides are thought to inhibit a general esterase not specifically connected with cholinesters.

Introduction

To explain the mode of action of some of the modern synthetic insecticides, it has been claimed that the phosphoric esters act as inhibitors of cholinesterase. The first symptoms observed in the toxic action of these types of poisons are paralysis and nervous disco-ordination. It seemed, therefore, reasonable to suspect that these substances interfered with synaptic transmission by inhibiting cholinesterase, particularly as these phosphoric esters are known to be powerful inhibitors of vertebrate cholinesterase. The present work deals with direct injection experiments designed to obtain evidence on the validity of this hypothesis.

Tobias, Killross & Savitt (1946) injected certain cholinesters into *Periplaneta americana*. They found acetylcholine toxic at 7–10 g./kg., carbachol at 1 g./kg., and acetyl-β-methylcholine non-toxic at 20 g./kg. Roeder, Kennedy & Samson (1947) show that di-*iso*propyl-fluoro-phosphonate (DFP) interferes characteristically with the transmission of nervous impulses across certain synapses in the nerve cord of *P. americana*. Immediate block of synaptic transmission has been obtained when DFP was followed by acetylcholine injection. Chadwick & Hill (1947) injected cockroaches with DFP, hexaethyl-tetra-phosphate (HETP) and eserine and found that, on a molecular basis, HETP was the most toxic. (Particularly so, one would assume, as the active part of the substance, tetraethyl-pyrophosphate (TEPP), formed only part of the HETP used.) The three substances inhibited cholinesterase *in vitro* in nerve cord brei, but HETP was less active than the others. Injection with eserine produced reversible inhibition of cholinesterase, whereas DFP inhibi-

tion was always irreversible. With HETP results were variable, but showed low inhibition compared with mortality. Although the authors were of the opinion that most of the toxic action of DFP could be accounted for by its anticholinesterase activity, no increase in mortality was observed when 100 μg. of acetylcholine or acetyl-β-methylcholine followed an injection of 5μg. DFP.

Roeder (1948) states that normal synaptic function in *P. americana* is dependent on the presence of a certain level of cholinesterase. Yet acetylcholine and related substances had no effect on transmission through the ganglia and added little, if anything, to the action of the cholinesterase inhibitors (HETP, DFP and eserine). Moreover, it was found that following the application of HETP, nerve cord contained 38 μg./g. of acetylcholine, and following eserine 32 μg. (by bioassay with frog nerves). Atropine, scopolamine and curare were without effect. The author concluded from the synaptic change following destruction of the enzyme that a synaptic mediator substance persisted, though he stressed that there is no evidence that the synaptic mediator was acetylcholine. As an explanation of these phenomena he postulated either that acetylcholine, atropine, etc., were unable to penetrate to the synapses, while cholinesterase inhibitors did, or that the synaptic transmission was not affected by acetylcholine.

Metcalf & Marsh (1949) found that bee brain could break down acetylcholine and tested the inhibition of the esterase by thirty-two organic phosphorus compounds which were at the same time tested as insecticides against flies and bees. Some of the compounds, notably parathion and TEPP, had a strong cholinesterase-inhibiting action, and, on the whole, there was good correlation between this inhibitory action and toxicity to insects. Atropine had no effect on injected cockroaches.

Dresden (1949), in experiments co-incidental to a study of the physiological action of DDT, injected cockroaches with tubocurarine but found no effect, although up to 300 μg. were injected. Krijgsman, Dresden & Berger (1950) studied isolated preparations of the cockroach heart. The insect heart is neurogenic, that is to say, acetylcholine does not inhibit it in low concentrations but stimulates it in high concentrations (nicotine-action). Both TEPP and acetylcholine were shown to have such an effect, and the heart could be sensitized to acetylcholine by TEPP concentrations too weak to have an effect themselves. Lord & Potter (1951) were unable to demonstrate an enzyme hydrolysing acetylcholine in breis of *Tribolium* and *Tenebrio*. These preparations did, however, hydrolyse *o*-nitrophenyl acetate and ethyl butyrate, and the hydrolysis could be inhibited by TEPP. They made the point that, since TEPP is an ovicide, acting on stages before the development of the nervous system, its action was likely to be unconnected with anti-cholinesterase activity. Tests by Diggle & Gage (1951) showed that pure parathion was not an *in vitro* inhibitor of the cholinesterase of rat brain, while the brain of rats killed with it showed no cholinesterase activity. The S-ethyl isomer of parathion, on the contrary, was a very powerful inhibitor, although somewhat less insecticidal than

parathion. It seemed likely that parathion was changed into an esterase-inhibiting substance in the body of the rat.

The most important points from these papers appear to be:

DFP, TEPP (HETP) and eserine are undoubtedly cholinesterase inhibitors and can act as such on insect nerve tissue, which has the ability to break down cholinesters, and can produce a substance that is like acetylcholine in its action on vertebrate nerve; but acetylcholine itself appears to have no action on the insect, nor is there any evidence that it plays any part in synaptic transmission. The only established physiological action by acetylcholine alone on the insect is a nicotine action on the heart.

In our experiments, here reported, we have endeavoured to study the effect of cholinesters on the African migratory locust—*Locusta migratoria migratoriodes* R. & F.—by direct injection into the body cavity of the insect. Substances that are known inhibitors of mammalian cholinesterases were also studied, and so was their interaction with acetylcholine. They were compared with TEPP, which is a very powerful insecticide, whose mammalian anticholinesterase activity is well known. In the mammal, atropine is the usual antidote against poisoning by cholinesterase inhibitors, and we have investigated its effect in the insect. Finally, we have included in our work two substances which have a powerful effect on the mammalian nervous system, *d*-tubocurarine and adrenaline.

The advantage of the injection technique for work of this type is that no problem arises with regard to penetration through the integument. Contact insecticides act as such not only because they are poisons, but also because they can penetrate to the site of action through the complicated structure constituting the insect's body wall. Some of the substances in which we were interested may not have this characteristic and might therefore appear non-insecticidal. Mixing all substances directly with the haemolymph of the insect by injection would appear to give the highest possible chance of access to the organs concerned.

Method

Young virgin adults of *L. migratoria migratoriodes*, bred at a temperature of approx. 30° C. on fresh grass or cereal shoots, were used. Equal number of each sex were used in all experiments.

Injection was carried out with an 'Agla' micrometer syringe, using a 26 B.W.G. exploring needle. For injection the locust was held ventral side upward, and the needle of the syringe, which was fixed on a retort stand, passed forward tangentially underneath the second abdominal tergite until its tip would be in the middle of the thorax, between the body wall and the alimentary canal. The insertion was made slightly laterally to avoid injuring the nerve cord. Mortality counts were made after 24 hr. All chemicals were dissolved in Ringer's solution with 1 % glucose unless otherwise stated in the tables. The total quantity injected varied according to doses, but never exceeded 0·05 ml. Controls were always injected with the solvent only at

its maximum quantity used in the experiment, and never gave significant kills. In experiments where there was injection with two substances, the second injection followed immediately on the first (with the exception of the experiments reported in Table 4*a*).

Results are expressed as μg. per locust. This corresponds approximately to mg./kg., the average weight of locusts of equal sex ratio being about 1 g. Individual locusts were not weighed, as a large part of their weight is made up by gut contents and skeletal structure, which vary considerably between individuals and cannot enter into the metabolism of the poisons. A better correlation of dosage and mortality is always obtained when using dosage per locust than when using dosage per weight of locust.

RESULTS

(*a*) *Toxicity of substances applied alone*

Table 1 summarizes all experiments that were undertaken to assess the toxic action of the chemicals tested. Of the four cholinesters tested none seemed to have any effect on the locust. They did not produce any significant mortality, nor were there any symptoms, temporary or permanent, to be observed on the treated insects.

TABLE 1. *The toxic action of cholinesters, mammalian cholinesterase inhibitors and other substances to the locust*

Name	Applied as	Result
Acetylcholine chloride (B.D.H.)	3 % in Ringer	No kill at 960 μg.
Acetyl-β-methylcholine (Savory and Moore)	1 % in Ringer	No kill at 160 μg.
Carbamyl choline (B.D.H.)	1 % in Ringer	No kill at 160 μg.
Benzoyl choline chloride (B.D.H.)	1 % in Ringer	No kill at 320 μg.
Choline chloride (B.D.H.)	3 % in Ringer	No kill at 1200 μg.
Prostigmine bromide (Roche Products)	3 % in Ringer	10 % kill at 1200 μg.
Eserine (physostigmine) (B.D.H.)	0·5 % in Ringer	M.L.D. 20–25 μg.
Tetraethyl pyrophosphate (Albright and Wilson)	0·05 % in Ringer	M.L.D. approx. 1·25 μg.
Diethyl-*p*-nitrophenyl-thiophosphate (Albright and Wilson)	0·02 % in 6 % diacetone alcohol in Ringer	M.L.D. approx. 2 μg.
d-Tubocurarine chloride (Burroughs Wellcome)	2·5 % in Ringer	No kill at 800 μg.
Atropine B.P. (Burroughs Wellcome)	2·5 % in 25 % ethyl alcohol in Ringer	35 % kill at 800 μg.
Adrenaline (B.D.H.)	1·5 % in special solvent*	No kill at 800 μg.

* Solvent for adrenaline: 1 % Na-metabisulphite and 0·8 % tartaric acid in water. 0·04 ml. of this solvent, injected into locusts produces narcosis lasting several hours, followed by complete recovery.

Prostigmine bromide was also non-toxic and produced no symptoms. Eserine (physostigmine), however, had a clear toxic activity and produced paralytic symptoms immediately after injection. In all cases where death did not occur, complete recovery from these symptoms took place within a few hours.

Atropine gave a kill of 35 % at 800 μg., but was non-toxic at all lower dosages tested. No symptoms of any kind were observed. *d*-Tubocurarine and adrenaline were non-toxic and produced no symptoms.

The two phosphorus insecticides were much more toxic than any other substance tested, and both caused immediate paralysis. With TEPP, complete recovery occurred invariably within a few hours in all locusts that did not die, while recovery from parathion poisoning was slower. This is probably due to the greater chemical stability of the latter substance.

(*b*) *Antagonistic action of atropine*

In these experiments, summarized in Table 2, injections of either TEPP or eserine were followed by injections of atropine. In one experiment the order of injection was reversed, and in another the TEPP was applied topically to the head instead of being injected.

TABLE 2*a*. *Mortality of locusts injected with atropine in* 20 % *alcohol in Ringer's solution, following treatment with TEPP in Ringer's solution*

TEPP (μg.)	Atropine (μg.)	Mortality (%)
Injected:		
5	50	100
5	—	100
2·5	50	86
2·5	—	85
1·25	50	55
1·25	—	48
Topical:		
2·5	100	53
2·5	—	51
1·25	100	21
1·25	—	23

TABLE 2*b*. *Mortality of locusts injected with atropine in* 20 % *alcohol in Ringer's solution, following injection with eserine in Ringer's solution*

Eserine (μg.)	Atropine (μg.)	Mortality (%)
50	50	96
50	—	99
25	50 (100)	54
25	—	55
12·5	50	5
12·5	—	0

In work of this kind, considerable variation between results must be expected, especially when the number of test organisms is small. In comparing mortalities produced by injection of the lethal substances with and without atropine, it will be seen that this variation is completely random, and that no correlation exists between mortality and the injection of atropine.

As shown in Table 3, there is no evidence that *d*-tubocurarine enhances the toxicity of eserine or TEPP.

Table 3. *The mortality of locusts injected with* d-*tubocurarine in Ringer's solution, following injection with TEPP or eserine in Ringer's solution*

No. locusts per treatment	Inhibitor	Quantity (μg.)	*d*-Tubocurarine (μg.)	Mortality (%)
40	TEPP	1·5	100	95
40	TEPP	1·5	—	74
40	TEPP	0·75	100	82
40	TEPP	0·75	—	86
40	Eserine	30	100	83
40	Eserine	30	—	95
40	Eserine	15	100	25
40	Eserine	15	—	20
40	—	—	100	0

(*c*) *The effect of acetylcholine chloride on TEPP-treated insects*

In the first group of experiments (Table 4*a*), acetylcholine was injected immediately following injection of TEPP. Although only a few seconds elapsed between the two injections, the locusts mostly showed the usual symptoms of TEPP poisoning when the second injection took place. However, the presence or absence of acetylcholine chloride did not affect the rate of reviving in any way, nor did it aggravate the symptoms.

In Table 4*b*, the cholinester was injected after time intervals varying between 0 and 60 min., but there was no correlation between this time interval and mortality.

Table 4*a*. *The mortality of locusts injected with* 0·02 % *TEPP, followed by* 2 % *acetylcholine chloride*

No. locusts per treatment	TEPP (μg.)	Acetylcholine chloride (μg.)	Mortality (%)
80	2·0	200	84
80	2·0	—	90
80	1·0	200	45
87	1·0	—	35
80	0·5	200	24
79	0·5	—	30
80	—	200	0

Table 4*b*. *Mortality of locusts injected with* 0·01 % *TEPP, followed by* 2 % *acetylcholine chloride after varying time intervals*

No. locusts per treatment	TEPP (μg.)	Acetylcholine chloride (μg.)	Time interval (min.)	Mortality (%)
81	1·0	200	—	47
79	1·0	200	0	58
83	1·0	200	15	44
82	1·0	200	60	60
40	—	200	—	0

Discussion

The results reported—our own as well as those of other workers—support the view that the physiological action of cholinesters in vertebrates is quite different from its action in insects.

Of the four cholinesters we injected, none seemed to have any effect on the locust. They did not produce any significant mortality, nor were there any symptoms, temporary or permanent, to be observed on the treated insects. Tobias *et al.* (1946) give toxicities of 7–10 g./kg. for acetylcholine and 1 g./kg. for carbachol and show acetyl-β-methylcholine to be non-toxic at 20 g./kg. in the cockroach. We are of the opinion that these figures can have no bearing on the elucidation of the cholinesterase-inhibition by insecticides. It seems impossible that such concentrations could be reached by the action of inhibitors. Within any range we can reasonably assume to be of importance, these esters can be regarded as non-toxic. For comparison, the L.D.50 by subcutaneous injection into rats are given by Goodman & Gilman (1941) as follows: acetylcholine, 250 mg./kg., acetyl-β-methylcholine, 750 mg./kg., carbachol, 4 mg./kg.

Prostigmine also was non-toxic. Eserine had a clear toxic activity, and led to paralytic symptoms immediately after injection. The *Pharmaceutical Pocket Book*, 1948, gives the minimum recorded fatal dose to adult man as 1/10 grain. Assuming the average weight of an adult to be 70 kg., this would work out as 0·093 mg./kg. Such data are, of course, of little use for comparison of toxicities, but if the M.L.D. was assessed at ten times that amount, the substance would still be about twenty-five times more toxic to man than to the locust. It is considerably less toxic than the pure TEPP by injection to the locust.

Levy (1945) gives the M.L.D. for atropine to mice as 83 mg./kg. intravenously and 520 mg./kg. intramuscularly. Assuming the former treatment to be more comparable to our method, the difference in toxicity would be by a factor of 10 or 20. Even by the intramuscular method, atropine would be only about half as toxic to insects as to mice.

With tubocurarine, differences are even more striking. Everett (1948) found the intravenous M.L.D. to mice to be 0·1–0·125 mg./kg., and Berger & Schwartz (1948), the intraperitoneal M.L.D. as 0·5 mg./kg. This compares with no effect at all both in the present experiments and those of Dresden (1949). The chief action of tubocurarine in the mammal is on the neuromuscular junction. It seems, in the absence of any symptoms, that here too a different physiological process is found in insects and mammals.

Gaddum (1949) gives the 'lethal dose' of adrenaline to the rabbit as 0·2 mg./kg. by intravenous and 15 mg./kg. by subcutaneous injection. We have failed to produce death or symptoms at a dose equivalent to 800 mg./kg. It seems, therefore, that the existence of adrenergic as well as cholinergic nerve processes is subject to doubt.

Nor does atropine seem to act as an antagonist to cholinesterase inhibitors in insects. In the five experiments reported, no evidence was found of a significant reduction in mortality. The variations encountered by the experimental method, as in all bio-assay work involving insects, are large, and must be held to be accountable for the differences shown in Table 2.

In mammals, atropine abolishes the muscarinic effects of acetylcholine, and its use to counteract poisoning by cholinesterase inhibitors is based on this property. If, as the present experiments suggest, acetylcholine itself has no effect on the insect, and the presumed cholinesterase inhibitors kill by other means than the prevention of the destruction of acetylcholine, it is to be expected that the mortality of insects would be unaffected by administration of acetylcholine, as is indeed the case.

The injection of acetylcholine into locusts previously paralysed by injection with TEPP has no demonstrable effect on subsequent mortality. It does not seem that acetylcholine plays any role in the process of which TEPP is an inhibitor.

There is also no evidence that tubocurarine has any enhancing effect on the toxicity of eserine and TEPP.

Three hypotheses may be put forward as a possible explanation of all these phenomena:

(1) The insect cholinesterase, although inhibited by extremely small quantities of TEPP, is powerful enough to decompose the large amounts of cholinesters injected instantaneously.

(2) The physiological action of acetylcholine is strictly localized, and injected doses of the cholinesters, atropine, tubocurarine and physostigmine cannot reach the site of action (synapses) even though the integument has been by-passed, whereas TEPP and eserine can.

(3) Acetylcholine and the other esters do not have a physiological effect on the insect (in particular as synaptic mediators) at all, and therefore the insecticidal action of TEPP, etc., cannot be one of cholinesterase inhibition. (The last two of these hypotheses have already been mentioned by Roeder (1948).)

The first hypothesis, we think, should be dismissed. Apart from the fact that such properties would be rare amongst enzymes, there are never any symptoms to be observed on injection of cholinesters, while injection of TEPP and eserine produce immediate paralysis and nervous disco-ordination. If these symptoms are due to an immediate accumulation of acetylcholine, some similar symptoms should at least temporarily be observed by injection of large amounts of this substance.

The second, which concerns differences of penetrative ability between the active and inactive substances, has some points in its support. Although the integuments cannot act as a barrier, injection into the abdominal cavity does not mean immediate access to the synapses. These are surrounded by an epineurium, about the nature of which little is known. Nevertheless, there seems to be no obvious reason supporting these assumptions from the point of view of purely physical properties.

If the site of action can be reached in the aqueous phase, neither the size of the molecule nor any physiological experience indicates that they should not do so. Admittedly, the lipoid solubility of the cholinesters, atropine and tubocurarine appears to be very low, and if a lipoid layer has to be passed to reach the site of action, this would be an impediment. But so is the lipoid solubility of eserine, and, although this substance is less toxic in insects than in vertebrates, it is yet very much more toxic than the cholinesters. The lack of data on mammalian toxicity makes it very difficult to arrive at definite comparisons.

Two observations in the literature reviewed give support to the anticholinesterase activity of insecticides, which, if proved, would leave differences in penetration power as the only possible explanation. First, there are the observations by Tobias *et al.* (1946) and Roeder (1948) on the accumulation of acetylcholine in the nerve cord of the cockroach after treatment with insecticides. But the only fact clearly established by these authors is the presence of a substance which acts like acetylcholine on the rectus abdominis of the frog. There is no evidence to show that the substance in question really was a choline ester. Secondly, there is the good correlation obtained by Metcalf & Marsh (1949) for the toxicity of their phosphorus compounds and their ability to inhibit the acetylcholinesterase of bee brain. Against this, however, must be quoted the finding of Chadwick & Hill (1947) that HETP was more toxic than DFP or eserine by injection, and less active as an *in vitro* inhibitor of cholinesterase. It may also be that the cholinesterase of bee brain plays no role in the synaptic transmission in that quality—in other words, that an enzyme fulfilling a function on a different substrate also happens to hydrolyse cholinesters.

The main support of the third explanation—that cholinesters do not act as synaptic mediators in insects—derives from the fact that no direct influence of cholinesters has been noted. The sole exception is the observation by Krijgsman *et al.* (1950) that acetylcholine has a stimulating effect on the heart. This effect alone would certainly be of little importance in insecticidal action. The insect heart does not have the important function of the vertebrate heart; it is an open system for the general, and not very efficient, distribution of body fluids with no respiratory function. It could be completely stopped long enough to allow for the excretion, not merely the enzymatic hydrolysis, of the acetylcholine to take place, without killing the insect.

Adoption of the third theory would postulate a synaptic mediator which acts similarly to acetylcholine on the nerve of the frog and is decomposed by an enzyme also capable of hydrolysing acetylcholine and of being inhibited by substances inhibiting cholinesterases. It must be a substance less physiologically specialized than acetylcholine, since it can produce actions in vertebrates similar to the latter compound, but not vice versa. The performance of the same physiological function by two different methods in two groups of organisms is not unusual, even where the two groups are much more closely related than insects and vertebrates.

In spite of the difficulties of these postulates, we feel that in the absence of sufficient evidence to arrive at definite conclusions, there are more weighty arguments at present for the third theory than for the second one. The absence of any effect of the injection of cholinesters is too striking to be ignored. We conclude, therefore, that it is doubtful whether insecticides do act as cholinesterase inhibitors. The enzyme concerned and inhibited by the phosphoric insecticides is more likely to be a general esterase, a view which receives support by the results of Lord & Potter (1951). It is hoped that an *in vitro* study of the activity and inhibiting properties of insecticides will form the subject of a further communication.

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INSECTICIDAL-ACTION STUDIES WITH BISDIMETHYLAMINO FLUOROPHOSPHINE OXIDE CONTAINING ^{32}PHOSPHORUS

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The absorption of the systemic insecticide bisdimethylamino fluorophosphine oxide containing ^{32}P by plants has been studied, and where possible comparisons have been made with bisdimethylaminophosphonous anhydride.

The radio-oxide is absorbed by the roots of a broad-bean plant from culture solutions. The level of radioactivity in the plant increases as the solution is absorbed and is higher in the washed roots than in the rest of the plant. The radioactivity of the remaining culture solution decreases as more of it is taken up by the plants showing that the roots selectively absorb the oxide from solution. In this latter respect it differs from the anhydride which is selectively rejected at similar rates of transpiration. The material is absorbed more slowly by plants growing in soil than in sand.

An appreciable part of the oxide absorbed by the roots is given off as vapour by the leaves. The transpired material can be collected and shown to be radioactive and systemically insecticidal. Aphids feeding on treated plants contain radioactive material.

The oxide is less lipoid soluble than the anhydride and does not penetrate as readily into the leaves of the broad bean. Since it is also lost by vaporization from the plants only small amounts are translocated following leaf applications to beans, cabbages and hops.

Introduction

Bisdimethylamino fluorophosphine oxide (hereinafter called oxide), like bisdimethylaminophosphonous anhydride (hereinafter called anhydride), is one of the materials discovered by Schrader and Kükenthal, and shown by them to possess systemic insectical properties. Generally speaking the oxide is more toxic to mammals, both more toxic to insects and toxic to a wider range of species and more phytoxic than the anhydride. It is also more volatile than the anhydride, and plants treated systemically with the compound give off vapour sufficiently rapidly to be capable of killing insects alighting on them by a purely fumigant effect. Since the oxide is lost in this way from the plant, it is less persistent than the anhydride and usually incapable of showing systemic action following slow absorption through leaves.

An attempt has been made to study the behaviour of the oxide in relation to the plant following the same methods as were employed for the anhydride (David, 1951). Unfortunately, owing to the poor yields obtained in synthesizing small

quantities of the radio-compound, it was felt desirable to discontinue its preparation and not all the tests which had been planned have been completed. Those reported illustrate the systemic properties of this compound and some of the ways in which it differs from the anhydride.

Materials and methods

The bisdimethylamino fluorophosphine oxide $[(CH_3)_2N]_2POF$ containing ^{32}P employed in the investigation was kindly prepared by Dr B. A. Kilby and Mr J. E. Gardiner, and was in general similar to the normal material used in earlier experiments (David & Gardiner, 1951). The methods of assay, the level of radioactivity and the general biological techniques were also the same as those used for the radio-anhydride (David, 1951). Because of the greater volatility of the oxide, however, a reflux condenser was used during extraction of the chopped plant material with boiling caustic soda. In preliminary experiments dilute solutions of the oxide were boiled under various conditions to determine how much of the oxide was likely to be lost in the extraction process. The results given in Table 1 show that when boiled in an open beaker in the presence of caustic soda the solutions lose very little activity but in practice a reflux condenser has been employed.

Table 1. *Loss of radio-oxide from boiled solutions*

	Concentration of solution	
	0·02 %	0·01 %
	Percentage remaining	
Treatment given		
None	100	100
Boiled/water	91·5	64, 69
Boiled/2 % NaOH	95·6	—
Boiled/8 % NaOH	—	92, 96
Refluxed/alone	—	98
Refluxed/8 % NaOH	94·0	97

The recovery figures obtained by extracting plants given a known dose by the cut tap-root technique (David & Gardiner, 1951) are difficult to interpret. Although the extractions were sometimes made in less than 1 hr. after the plant had absorbed its dose, that is within about 3–4 hr. of the beginning of the experiment, the recovery of oxide was never higher than about 70 %. With the quantity of radio-oxide available it has not been possible to investigate how much of the material unaccounted for was (*a*) left in the plant due to incomplete extraction, (*b*) lost by vaporization during the period allowed for absorption, (*c*) lost by the lateral roots to the water in which, it will be remembered, they were immersed. There is no evidence for this latter suggestion but it is included for the sake of completeness.

Results

Absorption by roots from culture solutions

When bean plants were placed in solutions of the oxide it was absorbed and distributed throughout the plants. The level of activity increased steadily throughout the course of the experiment, so long as the plant was not seriously injured. The concentration in the roots was always higher than in the aerial parts of the plant (Table 2).

Table 2. *The rate of accumulation of radioactive material by broad beans from solutions of the oxide*

(The figures given are the average of separate determinations on two plants.)

Plant no.	Time sampled (hr.)	Root assay (mg./kg.)	Bean assay (mg./kg.)	Top assay (mg./kg.)
1, 2	2	5·5	0·0	0·0
3, 4	5	35·5	0·0	20·1
5, 6	48	111	14·9	45·9
7, 8	72	183	20·0	127
9, 10	120	357	42·0	218

Table 3. *The absorption of the oxide by the roots of the broad bean (19 June 1950)*

(Vol. abs. = c.c. of solutions absorbed by plants from the 100 c.c. supplied; loss % = percentage change (loss) in activity of solution due to absorption by plants.)

		Days ... 2		4		7		9	
Source	Conc. (%)	Vol. abs.	Activity loss (%)	Vol. abs.	Activity loss (%)	Vol. abs.	Activity loss (%)	Vol. abs.	Activity loss (%)
Plant 1	0·01	30	15	48	22	73	37	82	39
Plant 2	0·01	21	16	40	25	62	28	75	31
Plant 3	0·005	20	36	34	47	63	65	87	64
Plant 4	0·005	23	37	37	46	66	63	93	67
Plant 5	0·001	21	24	36	36	68	42	89	47
Plant 6	0·001	26	33	46	56	90	33	—	—
Control	0·005	0	—	1	—	3	0	4	0
Standard	0·01	0	—	1	—	3	0	4	0

Table 4. *The absorption of the oxide—the total percentages of water and oxide removed by the roots*

	Days ... 2		4		7		9	
Source	Water (%)	Oxide (%)	Water (%)	Oxide (%)	Water (%)	Oxide (%)	Water (%)	Oxide (%)
Plant 1	30	41	48	55	73	83	82	89
Plant 2	21	34	40	55	62	72	75	83
Plant 3	20	49	34	65	63	87	87	95
Plant 4	23	52	37	66	66	87	93	98
Plant 5	21	30	36	59	68	82	89	84
Plant 6	26	51	46	72	90	93	—	—

By the end of the first day of the experiment the plants were free of aphids. It will be noted that at this time the radioactivity indicates a concentration of oxide in the plant well in excess of the 6·0 mg./kg. previously found to give a complete kill of aphids (David & Gardiner, 1951).

In this type of experiment it is not known whether the plants take up the oxide and water in the same ratio as they occur in the solutions. With the anhydride it has been shown previously that proportionately more water than anhydride was absorbed, so that the remaining solution became progressively more concentrated (David, 1951). On the other hand, with the oxide at similar rates of transpiration the solutions are weaker at the end of the experiment, as Tables 3 and 4 show. There is not sufficient evidence to decide what relationship, if any, exists between the absorption of the oxide and water, but it seems certain that the oxide enters plants more readily via the roots than the anhydride.

Absorption by the roots from sand and soil

In previous experiments solutions of the oxide were watered on to the soil and sand in pots in which plants were growing, and it was evident that aphids were killed at lower dosages and in shorter times in the case of plants in sand. This observation suggested that the oxide, like the anhydride, is most rapidly absorbed by the plants growing in sand. The plants were growing in $3\frac{1}{2}$ in. diameter fibre pots and were watered with 20 c.c. of an 0·05 % solution of the radio-oxide. When assayed at various intervals later, it was found that the radioactivity of those in sand was higher than those in soil (Table 5). It will be noted that the differences between the two are as great as those found for the anhydride. Since the oxide is also almost certainly lost more rapidly from the plants which attain the higher concentrations, it must be concluded that this effect is masked by the relatively more rapid absorption.

Table 5. *Radioactivity of aerial parts of plants growing in sand and soil treated with the oxide* (20 *June* 1950)

Day cut	Plant no.	From sand (mg./kg.)	Plant no.	From soil (mg./kg.)
1 (21. vi. 50)	1, 2	21	1, 2	1·5
2 (22. vi. 50)	3, 4	88	3, 4	9
4 (24. vi. 50)	5, 6	95	5, 6	9
6 (26. vi. 50)	7, 8	188	7, 8	10

The calculation of the oxide content is made on the assumption that no decomposition has occurred and is not corrected for incomplete recovery of the material from the plants.

Transpiration of radioactive material

In a previous paper it has been shown that plants treated with the oxide at the roots give off a toxic vapour from the leaves. This effect is apparent when all possibility of direct fumigant action from the material applied to the roots is excluded. It also is possible to collect the transpired material (David & Gardiner, 1951). This experiment has now been repeated using the radio-oxide. The equipment was exactly the same as that previously described. Three plants were placed in an 0·05 % v/v solution of the oxide, and the material condensing over the period 24–48 hr. later was collected.

TABLE 6. *The radioactivity and oxide content of material transpired by beans*

Experiment number and date	Volume of condensate (c.c.)	Percentage of oxide in condensate
1 (5. vi. 50)	18	0·0003
2 (12. vi. 50)	10	0·0018

TABLE 7. *The biological activity of the condensate against* Aphis fabae *on cut broad beans* (14 *June* 1950)

Condensate (%)	Calculated oxide content (%)	Weight of cut bean top (g.)	Percentage of aphids dead or moribund after 24 hr.
100	0·0018	4·9	87
50	0·0009	2·2	96
0	0	3·5	5

The second experiment in Table 6 was carried out with a more highly radioactive sample of the oxide than Exp. 1, and the results are likely to be more accurate. The condensate obtained in this experiment was used for biological tests with aphid-infested bean tops, again using the method previously described (David & Gardiner, 1951). The results are given in Table 7. The oxide content of the condensate in Tables 5–7 has been calculated on the assumption that all the radioactive material which it contained is the unchanged oxide. So far as can be judged, the biological effectiveness of the solutions bears out this assumption.

Penetration and translocation following application of the oxide to the leaves

It is well known that the anhydride is absorbed by the leaves of certain plants and translocated in insecticidal quantities (Ripper, Greenslade & Hartley, 1950; David, 1951). A similar effect has not been reported with the oxide, and indeed it seems possible that if absorption does occur through the leaves the material might be so

readily lost again by vaporization that it would not be transported very far from the point of absorption. What would happen must obviously depend on the relative rates and areas over which the two processes of absorption and evaporation take place.

Penetration

Before any substance can be translocated it must penetrate into the leaves. Determinations were therefore made of the quantities of oxide left on the surface of leaves and which had penetrated into them at different times after application. The method of washing the leaves and carrying out the assay were the same as those used for the radio-anhydride (David, 1951). If the results obtained, given in Table 8, are compared with those previously reported for the anhydride (see David, 1951, Table 10), it may be seen that the oxide is lost more rapidly than the anhydride from the dipped leaves. Also, after a time, most of the quantity remaining is still recovered in the washings and not in the extract of the leaves as in the case of the anhydride. This suggests that the oxide does not penetrate so rapidly into the leaves as the anhydride. An obvious explanation for this would be that the oxide is less soluble in the waxy layer on the leaves than the anhydride. This suggestion receives confirmation from the fact that the oxide is very much less soluble in olive oil than the anhydride.

Table 8. *Distribution of radioactive material between the surface and interior of seven pairs of bean leaves after dipping in a solution containing* 0·1 % *oxide and* 0·1 % *Teepol (wetter) and its persistence in the leaf*

Time after dipping (hr.)	Percentage of total recovered on the day		Percentage (of total on day 1) lost
	Washed off	Extracted	
1	84	16	0
5	80	20	15
24	72	28	37
96	75	25	41

Translocation

From the results just presented, it appears that some of the radioactive material penetrates into the leaf, or at any rate it cannot be removed easily by washing. It remains to consider whether there is any evidence of translocation. Experiments were carried out using plants of broad bean, cabbage and hop. Leaves selected for dipping were on the middle regions of the stems so that the plant carried older leaves below and younger leaves above the dipped leaves. The solution contained 0·1 % v/v of the oxide with 0·1 % w/v of Aerosol OT 100 as a wetter. The general treatment and methods were the same as those used for the anhydride.

The results obtained are summarized in Table 9, from which it can be seen that there was no evidence of translocation in the broad bean, that the hop plants showed relatively low levels of upward translocation and that in one of the plants there was apparently downward translocation also, and that one cabbage plant showed

evidence of upward translocation. Unfortunately there was insufficient radio-oxide to do an adequate number of confirmatory experiments, but it may be mentioned that essentially similar results had been obtained in a preliminary test, and that this also included strawberry plants, where translocation of the oxide also appeared to occur from older to younger leaves. It will be noted that the amount of oxide on leaves given repeated treatment did not increase to the same extent as with the anhydride. This may be because the oxide does not penetrate readily into the leaves and so would tend to get removed at each new treatment. This alone would reduce the proportion of material translocated in these experiments.

TABLE 9. *Translocation of radioactive material after application to the leaves* (19 *June* 1950)

					Radioactive material (mg./kg.)		
Plant	No.	No. of leaves treated	No. of times treated	Duration of experiment (days)	In leaves below treated leaves	In treated leaves	In leaves above treated leaves
Broad bean	1	4	13	5	<1	130	<1
	2	4	6	2	0	114	<1
Hop (OR 55)	1	4	13	4	7	343	7
	2	6	6	2	0	182	5
Cabbage	1	1	13	5	<1	63	5
	2	1	6	3	0	50	<1

Detection of radioactive material in aphids

Bean plants bearing large numbers of aphids were treated by the cut tap-root technique, and the falling aphids were collected in filter-paper collars. The radioactivities of the aphids and plants were determined according to the methods previously described (David, 1951). Table 10 shows that the radioactive material passes into the aphids. In considering these figures it should be assumed that the values given for aphids represent all the radioactive material which they actually contained, since the aphid-sodium hydroxide mixture was poured into the liquid-type Geiger-counter tube. The values given for the plants, on the other hand, are low by an unknown factor for reasons pointed out previously in this paper. The true values lie between those calculated and those determined. The difficulties which attend this experiment have already been discussed (David, 1951).

TABLE 10. *The occurrence of radioactive material in aphids which have fed on treated plants*

			Radioactive material (mg./kg.)	
Plant nos.	Conc. of solution (%)	Calc. conc. of oxide in plants (mg./kg.)	Plants	Aphids
1, 2	0·100	79	44	51
3, 4	0·050	53	33	36
5, 6	0·025	22	10	4

Discussion

The investigation described in this paper is regrettably incomplete but it shows that, as a systemic insecticide, the oxide differs in several respects from the anhydride.

Some time after applying the radioactive compound to the roots active material can be detected in all parts of the plants and rather later in the aphids feeding on them. In the case of the oxide, radioactive material is also given off by the leaves. This may be collected by condensation and can be shown to be systemically active. The condensate is presumed to contain unchanged oxide, although this has not been proved.

A consequence of this loss of toxic vapour is that insects merely sitting and not feeding on the leaves may be killed, as will be shown in a later paper, and also that the systemic effect will be less persistent than in the case of a less volatile compound. Both of these properties could have their practical application.

Bennett, Martin, Stringer & Woodcock (1949), using rooted willow sets and the brassy willow beetle (*Phyllodecta vitellinae* L.), found no evidence under the conditions of their experiments that the oxide was preferentially absorbed. In the present experiments, when plants were placed in solutions of the oxide, it was found that the solutions became weaker due to selective absorption of the oxide. With the anhydride at similar rates of transpiration, it will be remembered the solutions became more concentrated.

One other important difference between the oxide and anhydride has been noted. In comparison with the anhydride the oxide is less readily absorbed through leaves, probably because of its lower lipoid solubility. This property alone would prevent it acting efficiently when applied in this way. But it suffers from the additional handicap of being volatile, so that if absorption through the leaves is slow it may be lost again almost as soon and as quickly as it is absorbed. The results given in Table 9 support this suggestion.

I should like to thank Dr B. A. Kilby and Mr J. E. Gardiner for the radio-oxide and Mr B. O. C. Gardiner who assisted in the experiments.

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A TABLE OF ABBOTT'S CORRECTION FOR NATURAL MORTALITY

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In insecticide tests and other quantal assays, it is common practice to observe a batch of untreated material so as to allow for a non-zero response when no treatment is applied. When such a response occurs, the observed responses to the different doses can be adjusted by a formula usually known as Abbott's correction; briefly the argument is that if P_0 % is the response in the controls and P' % the true mortality due to a treatment, the latter will affect the remaining $(100-P)$ % of the test subjects, giving an observed response of

$$P = P_0 + \frac{1}{100} P'(100 - P_0),$$

so that

$$P' = P - P_0 \frac{100 - P}{100 - P_0}.$$

The accompanying table gives the values of the second term in the expression for P' for response in the controls of 1–25 %, i.e. the correction to be subtracted from the observed P to obtain the adjusted P'. The table is arranged as a critical table; to use it, find in the column headed by the control response the values that bracket the observed response, and use the correction opposite them in the left- or right-hand columns.

A table of Abbott's correction

Response in controls (%)

Correction	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Correction
0	50	76	84	88	91	93	94	95	95	96	96	97	97	97	97	98	98	98	98	98	99	99	99	99	99	0
1	.	27	52	64	72	77	81	83	85	87	88	90	90	91	92	93	93	94	94	94	95	95	95	96	96	1
2	.	.	20	40	53	61	67	71	75	78	80	82	84	85	86	87	88	89	90	90	91	92	92	93	93	2
3	.	.	.	16	33	46	53	60	65	69	72	75	77	79	81	82	83	85	86	86	87	88	89	89	90	3
4	.	.	.	.	15	30	40	49	55	60	64	68	70	73	75	77	78	80	81	82	84	85	85	86	87	4
5	.	.	.	.	.	14	27	37	45	51	56	60	63	67	69	72	74	75	77	78	80	81	82	83	84	5
6	.	.	.	.	.	.	14	26	35	42	48	53	57	61	64	66	69	71	73	74	76	78	79	80	81	6
7	.	.	.	.	.	.	1	14	25	33	40	46	50	54	58	61	64	66	69	70	72	74	75	77	78	7
8	.	.	.	.	.	.	.	2	15	24	32	38	44	48	52	56	59	62	64	66	69	70	72	74	75	8
9	.	.	.	.	.	.	.	.	4	15	24	31	37	42	47	51	54	57	60	62	65	67	69	70	72	9
10	.	.	.	.	.	.	.	.	.	6	16	24	30	36	41	45	49	53	56	58	61	63	65	67	69	10
11	.	.	.	.	.	.	.	.	.	.	7	16	24	30	35	40	44	48	51	54	57	60	62	64	66	11
12	.	.	.	.	.	.	.	.	.	.	.	9	17	24	30	35	39	44	47	50	53	56	59	61	63	12
13	.	.	.	.	.	.	.	.	.	.	.	2	10	18	24	30	35	39	43	46	50	53	55	58	60	13
14	.	.	.	.	.	.	.	.	.	.	.	.	3	11	18	24	30	34	39	42	46	49	52	55	57	14
15	.	.	.	.	.	.	.	.	.	.	.	.	.	5	12	19	25	30	34	38	42	46	49	51	54	15
16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7	14	20	25	30	34	38	42	45	48	51	16
17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	9	15	21	26	30	35	38	42	45	48	17
18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	10	16	22	26	31	35	39	42	45	18
19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	12	17	22	27	31	35	39	42	19
20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7	13	18	23	28	32	36	39	20
21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	9	14	20	24	29	32	36	21
22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	10	16	21	25	29	33	22
23	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6	12	17	22	26	30	23
24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	8	14	18	23	27	24
25	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	10	15	20	24	25
26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	7	12	17	21	26
27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	8	13	18	27
28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	10	15	28
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	7	12	29
30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	9	30
31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	6	31
32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	32

To adjust an observed % response: use the column headed by the response in the controls, find the values that bracket the observed % response and subtract the correction opposite them in the left- or right-hand columns. In doubtful cases use the *smaller* correction.

Examples:

Natural mortality, 7 %, observed kill 44 %, correction 4 %
12 % 17 % 11 %
4 % 40 % 2 %

PETROLEUM OIL FRACTIONS AS PLANT SPRAY MATERIALS

THE EFFECT OF REFINING AN OIL UPON ITS DEGREE OF PHYTOTOXICITY

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Data are presented showing the effect of degree of refining upon the specific gravity, viscosity, unsulphonatable mineral residue (U.R.), refractive index, average molecular weight, group analysis, sulphur content and phytotoxicity of two spindle oil distillates in the boiling range 300–390° C.

Cucumber plants proved very suitable for demonstrating differences in phytotoxic properties of the oils.

Spindle oils refined to a U.R. of 82 or higher, and a specific gravity of 0·885 or lower, showed no phytotoxicity in the tests on these plants. A fair agreement was found between the results of these tests and the specifications laid down by Dr H. Martin for spray oils.

Introduction

Large quantities of petroleum oils are used annually as insecticides and acaricides by commercial fruit growers. These oils are highly effective against certain pests, such as European red mite (*Metatetranychus ulmi* Koch) and scale insects of citrus.

Mineral oils are usually mixtures of the paraffins or alkanes (saturated chain hydrocarbons), the naphthenes (saturated cyclic compounds) and the aromatics (fully conjugated unsaturated 6-ring compounds). Of recent years a laboratory technique has been developed (van Nes & van Westen, 1951) which renders it possible to obtain a figure for the average percentages of carbon atoms present in each of these three groups, together constituting the total of the hydrocarbon molecules in an oil. This procedure is particularly valuable from a comparative point of view, since it allows oils to be classified into types rich or poor in naphthenes and aromatics. By this method it was confirmed that mineral oils from different origins can differ considerably in their content of the various constituting groups.

In England the specifications (Table 1) laid down by Martin (1935) for oils to be used as winter or summer sprays have been generally accepted, and in 1949 they

* This publication was prepared by T. Swarbrick from a report by J. W. Heringa and collaborators on work carried out in the above laboratory.

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were officially adopted by the Ministry of Agriculture and Fisheries, with some alterations of minor importance.

Table 1. *Specification of oils, according to Martin*

	Winter oil (grade E)	Summer oil (grade G)
Specific gravity (60° F.)	0·860–0·920	0·860–0·920
Boiling range (° C.):		
Not less than 90 % of the oil should distil above	315	300
Not less than 10 % of the oil should distil below	—	330
Not less than 50 % of the oil should distil above	350	340
Not less than 50 % of the oil should distil below	—	365
Not less than 20 % of the oil should distil above	380	370
Not less than 80 % of the oil should distil below	—	390
Viscosity Redwood I (70 °F.) (sec.)	125–500	75–150
Viscosity centistokes (21° C.) (cS.)	30–120	17–35
Unsulphonated residue minimum	60%	90 % vol.

These specifications of base oils for horticultural sprays, as in use to-day, are based on specific gravity, boiling range, viscosity, and percentage of unsulphonatable hydrocarbons.

They do not directly take into account the above-mentioned variation in the chemical composition of the oils, although the latter have a distinct influence on the four properties specified.

Unfortunately, isolated complaints of damage to plants by oil are reported every year. The incidence of damage has been sporadic and unpredictable, but the fear of damage is the main limiting factor in a wider use of petroleum oils as spray materials.

Summing up, it can be stated that although it has been possible to lay down certain practical standards for the base oils used in horticultural sprays from the results of a large number of investigators, these standards have so far been essentially empirical. A further development in petroleum oil washes is to be expected only when more knowledge on the problem of phytotoxicity has been collected. Systematic research was therefore started in this laboratory, and the present paper is the first of a series dealing with phytotoxicity in relation to the composition of mineral oils.

Materials and methods

Base oils

This investigation was carried out on the spindle oil fractions from two crude oils of different geographic origin. Both of these oils were known to be rich in naphthenic and aromatic groups. To prevent decomposition of the oil due to cracking at higher temperatures they were prepared by distillation of the crude oils with steam combined with vacuum so that they had approximately the same boiling

TABLE 2. *The effect of refining treatments upon the composition of two spindle oil distillates of naphthenic origin*

	Specific gravity (15/4° C.)	Viscosity (cS. at 20° C.)	U.R.	Average mol. wt.	Refractive index (n_D 20° C.)	Sulphur content (%)	Percentage of carbon present in			Phytotoxic effect upon cucumber plants
							Chains	Saturated rings	Aromatic rings	
Spindle oil distillate A	0·922	60	59	276	1·5138	1·86	45	34	21	High
1st furfural extract	1·034	275	14	265	—	4·16	—	—	—	Very high
2nd furfural extract	1·001	253	25	275	—	3·67	—	—	—	Very high
3rd furfural extract	0·985	203	29	280	—	2·94	—	—	—	Very high
Furfural raffinate from spindle oil distillate	0·889	53	76	307	1·4882	0·93	52	38	10	Slight
Furf. raff. treated 1 with fum. sulph. acid	0·884	52	82	297	1·4864	0·48	56	36	8	None
,, ,, 2 ,, ,,	0·878	48	88	—	—	0·22	—	—	—	None
,, ,, 3 ,, ,,	0·873	45	91	291	1·4787	0·11	58	40	2	None
,, ,, 4 ,, ,,	0·869	43	95	–	—	0·06	—	—	—	None
,, ,, 5 ,, ,,	0·866	41	96	297	1·4740	0·03	58	42	0	None
,, ,, 6 ,, ,,	0·865	40	97	—	—	0·01	—	—	—	None
Spindle oil distillate B	0·933	59	55	260	1·5206	0·39	44	36	20	High
1st furfural extract	1·030	193	12	250	—	0·89	—	—	—	Very high
2nd furfural extract	1·011	154	24	260	—	0·85	–	—	—	Very high
3rd furfural extract	0·990	139	32	290	—	0·76	-	—	—	Very high
Furfural raffinate from spindle oil distillate	0·898	58	73	298	1·4928	0·20	49	42	9	Slight
Furf. raff. treated 1 with fum. sulph. acid	0·894	57	76	290	1·4895	0·09	49	46	5	Very slight
,, ,, 2 ,, ,,	0·890	56	84	—	—	0·07	—	—	—	None
,, ,, 3 ,, ,,	0·885	53	90	286	1·4834	0·04	52	48	0	None
,, ,, 4 ,, ,,	0·882	50	93	—	—	0·03	—	—	—	None
,, ,, 5 ,, ,,	0·880	48	96	300	1·4796	0·02	52	48	0	None
,, ,, 6 ,, ,,	0·879	47	96	294	1·4788	0·01	—	—	—	None

range, viz. about 300–390° C. under atmospheric pressure.* By this means a more or less typical distillate in the spindle oil range was obtained. This was the starting material; it is called in this paper 'spindle oil distillate'. This spindle oil distillate was subjected to a progressive refining treatment (see Table 2). The first steps consisted of three successive extractions with an amount of furfural equal to the volume of distillate undergoing treatment. The fractions of material extracted in this way were recovered and freed from furfural by distillation, and are designated as furfural extracts I, II and III respectively. They consisted largely of material containing a high percentage of carbon atoms in aromatic rings. These three extractions removed about 34 % of the original distillate. That part of the distillate which remained after the three successive extractions is called the furfural raffinate.

Part of the furfural raffinate was further refined by subjecting it to six successive treatments, each with one-tenth of its volume of fuming sulphuric acid (20 % SO_3 content). The acid-treated oil was freed from acids by washing it with alcohol and water, and neutralized in the manner usual for this method of oil refining. This treatment removed a further 17–18 % of the original spindle oil distillate.

The result was that from each of the two base oils eleven products were obtained for testing. These were: spindle oil distillate, first, second and third furfural extracts, furfural raffinate, and the latter raffinate, treated 1, 2, 3, 4, 5 and 6 times respectively with one-tenth of its volume of fuming sulphuric acid. It will be seen later that the refining treatment causes them to differ from each other in many respects.

Each of the above eleven products from each of the two base oils was then emulsified by a standard procedure, based on the use of casein and a spreader and the resulting concentrated emulsion, containing about 75 % of oil, was diluted so as to contain 1 % of the concentrated emulsion, i.e. 0·75 % of actual oil. This 1 % emulsion was applied to test plants in a manner described below.

Biological materials and spraying methods

Cucumber was used as the test plant for the evaluation of damage caused by the oil fractions. This plant is extremely sensitive to oil damage, it differentiates clearly between the 'acute' and 'chronic' types of injury commonly encountered in this type of work, and plants of approximately the same age and size can be produced under glasshouse conditions all the year round, although artificial light may be required to supplement normal daylight during November to March. Cucumber plants just developing their sixth true leaf were used as standard test plants.

When necessary or convenient, supplementary trials were made on apple seedlings grown from seeds sown in the greenhouse and subsequently reared in pots.

The 1 % emulsions of the test materials were applied by means of a small hand sprayer employing a constant pressure of 3 atmospheres. The nozzle was of the

* ASTM distillation D 447–41; a drawback of this method is the probability of decomposition of the oil due to cracking at the high temperatures employed.

type used in knapsack sprayers. A special push-button release, giving immediate control of the spray, was employed.

The test plants were sprayed until thoroughly wetted. Each leaf was treated individually, care being taken to cover both the upper and lower surface equally. Observations were continued for a period of 14 days following treatment. Photographic records were obtained as required. Oils which caused no damage in duplicate trials were regarded as being non-phytotoxic under these conditions.

Experimental results

The numerical data collected during the course of this investigation are given in Table 2. The following are the main points of interest.

Effect of degree of refining upon phytotoxicity

The data show that both of the unrefined spindle oil distillates were highly phytotoxic. Treatment by three successive extractions with equal volumes (100 %) of furfural resulted in a raffinate which was still slightly phytotoxic. One or, at most, two treatments of this furfural raffinate with 10 % by volume of fuming sulphuric acid rendered it non-phytotoxic.

The furfural extracts proved to be very highly phytotoxic. The present technique also gave definite indications that the first of these extracts was more phytotoxic than the succeeding ones.

*Effect of degree of refining upon unsulphonatable mineral residue**

The U.R. (unsulphonatable mineral residue) of an oil is an index of its content of saturated compounds. It is therefore used to indicate the degree of refining of mineral oils.

The data given in Table 2 show that the unrefined spindle oil distillates had a U.R. of 55 and 59 respectively, but that this increased to 73 and 76 by the three successive extractions with furfural. Six successive treatments of this furfural raffinate with fuming sulphuric acid raised its U.R. to 96–97.

The data show that the furfural raffinate of the oils under investigation with a U.R. of 73–76 was still slightly phytotoxic to cucumber under test conditions, but that oils treated with sulphuric acid to a U.R. of 82–84 were no longer phytotoxic. It appears, therefore, that the limit of toxicity towards cucumber plants in the case of the spindle oils used is between U.R. 76 and 82 or roughly about 80, whereas oils with a U.R. above 82 could be regarded as safe in this respect.

Effect of refining on percentage of carbon in different groups

The refining of the oil results primarily in the removal of the carbon in aromatic ring structure, while the ratio of carbon in aliphatic chains to that in saturated naphthene rings did not change materially. The reduction in aromatic rings

* ASTM Method D 483–40.

obtained correlates with a decrease in phytotoxicity. From these few results it may be concluded that the raffinates with 9–19 % of the carbon in aromatic bonds were slightly phytotoxic under the test conditions employed, while those with 91 % or more were very injurious to cucumber plants.

Effect of refining upon specific gravity, viscosity and refractive index

The data given in Table 1 show very clearly that the products obtained in successive refining treatments of the oils under investigation progressively decrease in specific gravity, viscosity and refractive index.

It is important to note that the effect upon these two properties differs in intensity. Thus, specific gravity is reduced by about 6 % as the result of three extractions with furfural, followed by six treatments with fuming sulphuric acid, whereas the viscosity is lowered by about 20–30 % of its original value.

It should be noted, therefore, that the material which is removed by the furfural has a very high viscosity and a relatively high specific gravity.

Furthermore, it is shown that while the removal of some 34 % of the spindle oil distillate by extraction with furfural reduces its viscosity by about 12 %, further treatment six times with fuming sulphuric acid, which removes only a further 17 % of the distillate, reduces its viscosity by a further 18–20 %. It must be inferred, therefore, that the fractions removed by reaction with sulphuric acid had an even higher viscosity than those removed by furfural, and it is somewhat surprising to learn that an unrefined distillate in this relatively low boiling range, with a viscosity of 60 centistokes, may contain as much as 50 % of material with a viscosity of three to four times this figure.

Effect of refining upon average molecular weight

It is shown that, besides decreasing viscosity and specific gravity, furfural refining also results in an increase in the average molecular weight of the raffinate. Subsequent acid-refining of the furfural raffinate has practically no effect upon its average molecular weight. Quite clearly, therefore, the two refining methods, when used in sequence as in these investigations, both remove materials having a very high viscosity and a relatively high specific gravity; furfural, however, removes materials with a relatively low molecular weight, whilst acid eliminates products with a molecular weight not very different from that of the refined product.

Effect of refining upon sulphur content

The two spindle oil distillates used as base material differed widely in sulphur content, but both are almost totally freed from sulphur by the successive refining treatments.

Discussion

The data presented in this paper are part of those obtained during investigations aimed at providing safe and effective spray oils. The initial objective of this work was to determine whether a correlation exists between those characteristics of an oil which can be determined by modern techniques and its known phytotoxicity or insecticidal efficiency. With this information in hand it was hoped that it would be possible to select the best possible spray oil within the prevailing economic framework. In order to present the issues as clearly as possible it has been decided to discuss only phytotoxicity and not insecticidal value in the present paper.

The data now published on tests with oils in the spindle oil range show that although several characteristics (refractive index, sulphur content, molecular weight) of an oil show alterations in the course of refining, they are not suitable as a basis for specifications, as the limit of phytotoxicity does not lie at constant values of these magnitudes. On the other hand, oils with a U.R. of 82 or better were not phytotoxic under the prevailing test conditions and those with a specific gravity of 0·885 or lower would generally be regarded as safe. Unsulphonatable mineral residue gives a much clearer demarcation between oils which are, and those which are not, phytotoxic; on one occasion an oil of a pronounced paraffinic type, with a specific gravity of 0·872, was found to have a marked phytotoxicity.

The method of evaluating spray oils by applying them to young cucumber plants was originally devised as a test method for summer oils. The use of this method for testing winter sprays might at first sight appear somewhat remote from practice, and it might be argued that it is of little value on this account. Experience over many years, during which many oils have been examined and compared by several methods, leads us to the conclusion that it is quite impracticable to simulate field usage and to compare the phytotoxicities of different oil fractions in winter sprays, as the results then are hardly reproducible and differ considerably in comparable tests. Given a sufficiently broad background of working experience, the results obtained by the 'Cucumber technique' can be interpreted with a high degree of certainty and assurance. The results obtained on cucumbers have been occasionally verified by the use of apples, plums, etc., grown in pots and in the experimental garden.

It was of great interest to the authors to learn how far their results corroborate Martin's specifications. As these specifications are based on a considerable mass of practical experience, an important discrepancy might indicate that the proposed method of test is useless for assessing spray oils.

The spindle oils used as initial material in the present investigation were so chosen that their boiling range fell within the limits given in Martin's specification for spray oils. The refining processes used to treat these oils did not involve distillation, so that no appreciable change in boiling range can have resulted. The refined products obtained satisfied the requirements for winter sprays, but were

somewhat too viscous for summer oils. The latter are generally manufactured from a somewhat more volatile initial material.

Martin's limits for specific gravity are so wide (0·860–0·920) that they are met not only by the non-phytotoxic oils in the present investigation but also by oils which show a slight phytotoxicity. Highly phytotoxic oils, however, have a specific gravity above 0·920 and are excluded.

As regards U.R., the specification for winter oils (U.R. > 60) again excludes the highly phytotoxic oils in the present test series, as their U.R. was lower than 60. Oils which were slightly phytotoxic towards cucumber leaves, however, complied with the specification in this respect. The specification for summer oils (U.R. > 90), on the other hand, does not admit any oil which was phytotoxic in the authors' tests and would even have ruled out one or two non-toxic oils, though most of these meet the specification.

The results obtained with the cucumber-plant technique and the results from practice which are summarized in Martin's specification hence agree in so far that oils satisfying the winter specification were slightly phytotoxic according to the cucumber technique, whilst those complying with the specification to summer oils were non-phytotoxic. Both criteria would discard the highly phytotoxic oils as falling outside any specification.

It cannot be overemphasized that the present discussion is confined to phytotoxicity as displayed by oils prepared from base materials falling in the range of the so-called spindle oils. These spindle oils comprise the initial materials currently employed in the preparation of winter washes. It does not necessarily follow that the spindle oils give the best combination of a low toxicity to plants and a satisfactory insecticidal effect. Work in progress in the laboratory of one of the authors suggests that sprays made from fractions falling outside the spindle oil range may be considerably less phytotoxic.

The authors are indebted to Dr P. C. Blokker and the late Dr J. Ph. Pfeiffer of the Koninklijke/Shell-Laboratorium, Amsterdam, for their direction and interest in these investigations, and to Messrs Ph. Gerolt, M. W. S. Schuhmacher and A. C. van Beest for their assistance in this work.

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INVESTIGATIONS INTO THE HOST RANGES OF *DITYLENCHUS DESTRUCTOR* AND *D. DIPSACI*

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(With Plate 10)

Observations and inoculation experiments on the host ranges of *Ditylenchus destructor* and of a narcissus race of *D. dipsaci* have given results which may be tabulated as follows:

Host	Parasite: First record	Parasite: Present findings
Humulus lupulus	*D. dipsaci*—Percival, 1895	*D. destructor*—only
Syringa vulgaris	*D. dipsaci*—Steiner, 1927	*D. destructor*—only
Tigridia pavonia	*D. dipsaci*—Steiner & Buhrer, 1935	*D. destructor* and *D. dipsaci*
Begonia tuberhybrida	*D. dipsaci*—T. Goodey, 1929	*D. dipsaci*
Gladiolus hybridus	*D. dipsaci*—T. Goodey, 1929	*D. destructor* and *D. dipsaci*
Tropaeolum polyphyllum	*D. dipsaci*—Hastings, 1940	*D. destructor*
Stachys palustris	*D. destructor*—a new host record	

The host list of the stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev, of T. Goodey (1940) includes hop *Humulus lupulus* L. and lilac *Syringa vulgaris* L. In both plants the parasites are reported from the roots only. Thorne (1945) showed that an eelworm, which had previously been regarded as a biologic race of *Ditylenchus dipsaci* attacking potato tubers, was a separate species, *D. destructor*. Further hosts of *D. destructor* were reported by Hurst (1948), Goodey & Goodey (1949) and J. B. Goodey (1950, 1951). In all cases the attack was confined to the underground parts of the plant.

The infestation of *Tropaeolum polyphyllum* tubers by *Ditylenchus dipsaci* and its transfer to potato tubers was reported by Hastings (1940).

The cases of parasitism of hop, lilac and *Tropaeolum polyphyllum* were therefore re-examined, and experiments were also carried out with *Begonia*, *Tigridia* and *Gladiolus* to see whether *Ditylenchus destructor* only or both it and *D. dipsaci* were capable of attacking these plants.

Hops

Percival (1894, 1895*a*, *b*) discovered two different kinds of eelworms associated with diseased roots of hop when investigating the disease of 'Nettle-headed' or 'Skinkly' hops. These eelworms were *Heterodera schachtii* A. Schmidt (now *H. humuli* Filipjev) and the stem eelworm *Tylenchus devastatrix* Kühn (now *Ditylenchus dipsaci*). He noted that the stem eelworm had not been found before in roots and wrote: 'The *Tylenchi* occur in the thicker parts of the roots and live within the

cortex, destroying it, and making their way close up to the living bast. They are not uniformly distributed all over the root, but only in isolated patches, and contrary to their usual behaviour when met with in stems and leaves of plants, produce no excessive growth of tissue in their immediate neighbourhood. Where they are present the cortex loses its bright orange colour, decay sets in, and the root at that point is generally found to have an eccentric development of the woody rings of growth. The cells round about the parasites die, turn brown, and become disorganized; thus small spaces are formed in the living cortex. Many of the cells become filled with a dark-brown resinous substance.'

Both de Man and Ritzema Bos confirmed Percival's identification of the *Tylenchi*, though Ritzema Bos had some doubts at first, finding the nematodes smaller than usual but with no other constant differences.

In 1925, Duffield showed conclusively that there was no connexion between eelworm infestation by either *Heterodera schachtii* or *Ditylenchus dipsaci* and 'Nettlehead' of hops.

In 1939, Dr T. Goodey received diseased hop roots from Dr Ware of Wye. They came from a hop garden near Sittingbourne, Kent, where hundreds of hills showed weak shoots only a few inches long, the healthy hills having bines up to 12 ft. long. The hop set was partly or wholly rotted, and although the roots were generally strong and well developed they were black and rotting close to the set. Goodey (private communication) found considerable numbers of *D. dipsaci* in brownish lesions in otherwise healthy cortical root tissue. The diseased roots were preserved, and in September 1949 the writer re-examined some of this material. The eelworms dissected out had all the morphological features of *D. destructor*. Accordingly, an experiment was set up in which pieces of clean hop stool and hop roots were planted in pots of John Innes compost inoculated with *D. destructor* from potato. Seven months later one root, when split longitudinally, revealed extensive cortical injury from which many tuber eelworms were recovered. The cortical damage was similar to that reported by Percival and that exhibited by the 1939 material. The cortex was not bright orange in colour as described by Percival but that coloration may have been a peculiarity of the variety with which Percival was working.

The diseased root was cut up into small pieces and used to inoculate a pot in which a clean potato was planted. After 3 months' growth, nineteen new potatoes had been produced, of which fifteen had lesions, some quite extensive. In these lesions and in others on the potato haulm many *D. destructor* were found.

Concurrently with these experiments, attempts were made to obtain fresh material from the same hop garden at Sittingbourne. Specimens of weak plants were obtained early in July 1950 through the kindness of Mr H. H. Glasscock. The cortex of many of the roots was dark brown and showed typical symptoms of damage by *D. destructor*, many of which were present.

With this material as inoculum, an experiment was set up in which three pots were planted with potatoes, three with Spanish iris bulbs and three with rhizomes

of *Mentha arvensis*. At the same time, six pots were planted with clean hop root-stock; three being inoculated with *Ditylenchus destructor* from iris and three with mint rhizome material already carrying an infestation of *D. destructor* derived from iris bulbs.

The general plan and results of the two experiments are set out in Tables 1 and 2. The only infested iris material available when the experiment was set up was only very lightly infested and proved insufficient to cause perceptible damage to the hops. One potato, after 3 weeks' growth, had a stunted shoot, the leaves being rounded and distorted, very similar to the condition shown in fig. 3 of Seinhorst (1949). Tuber eelworm was found in lesions on the potato haulm and in new tubers; gravid females were recovered from lesions on both the mint rhizomes and hop roots.

TABLE 1. *Transfer of* Ditylenchus destructor *from hop to potato, iris and mint*

	Plants tested		
Source of inoculum	Potato	Iris	Mint
Hop roots	*d* + +	+ + +	+ + +

d = tuber rotted; + = infestation.

TABLE 2. *Transfer of* Ditylenchus destructor *from iris and mint to hop*

Source of inoculum	Hop as test plant
Iris bulbs	− − −
Mint rhizome	+ + −

+ = infestation; − = no infestation.

LILAC

Steiner (1927) reported finding *D. dipsaci* in the roots of *Syringa vulgaris*; remarking that as far as he knew it was the first occasion that the parasite had been reported from roots. He was apparently unaware of Percival's findings in hop roots (1894). In September 1949 some small lilac plants were potted with a liberal inoculum of *Ditylenchus destructor* from potato. Six months later these plants were turned out, the soil carefully washed away and the roots examined. Several pieces of root had dark brown lesions containing numerous *D. destructor* in all stages of development. The diseased roots were removed, cut into small pieces and used to inoculate a pot in which a clean potato was planted. After 3 months' growth one new tuber had a small lesion which yielded adults, larvae and eggs of *D. destructor*.

Another experiment was set up at the same time with small lilac plants and inocula consisting of dried infested material containing populations of *D. dipsaci* from narcissus, red clover, oats and teazle, and one consisting of soil carrying a population of stem eelworm from onions. One plant was grown as a control. Four months later each plant was carefully washed free of soil and the roots searched for lesions. There were some dead roots on all the plants but no eelworms could be found in any of them. The soil from each pot was also carefully sieved

by Thorne's sieving technique (T. Goodey, 1949). From the pots containing the oats, onion, narcissus and teazle inocula some *D. dipsaci* were recovered. These nematodes were either pre-adult or adults; no larvae younger than pre-adult were seen. None of the females had been spermatized nor was a single gravid one found. Most of the adults were moribund and had lost nearly all their dense, granular storage materials from the gut walls. It was obvious that no infestation of the lilac roots had taken place, that no reproduction had occurred and that, in all probability, the eelworms had starved.

Tigridia, *Begonia* and *Gladiolus*

Ditylenchus dipsaci was reported by Steiner & Buhrer (1935) from bulbs of tigridia imported from Holland, and also (Anon., 1936, 1937) in bulbs intercepted within the United States. T. Goodey (1929) recorded stem eelworm in the tubers of *Begonia tuberhybrida* and corms of *Gladiolus hybridus*.

Bulbs of *Tigridia pavonia*, tubers of *Begonia tuberhybrida* and corms of *Gladiolus hybridus* were bought early in 1949. Each specimen was examined carefully for disease, the tunics being stripped where necessary. The begonias and gladioli were clean but from two of the tigridia bulbs, which showed some dark streaks on the lower parts of the tunics, three males and one female *Ditylenchus destructor* were obtained. The two bulbs from which these eelworms were obtained were grown in the tigridia control pots. Table 3 shows the plan and summarizes the results of the experiments. The inocula used were dried narcissus leaves carrying *D. dipsaci* and pieces of potato tuber infested with *D. destructor*. The tigridia and begonias were grown in the greenhouse and the gladioli were put into an outside gravel plunge.

Table 3. *Transfer of* Ditylenchus dipsaci *and* D. destructor *to* Tigridia, Gladiolus *and* Begonia

	Plants tested		
Inoculum	*Tigridia*	*Gladiolus*	*Begonia*
D. dipsaci (narcissus)	*d* − + + (+)	− + + + +	+ +
D. destructor (potato)	*d* + (+) (+) (+)	− − + + +	? (?)
Control	− −	− −	− −

− = remained clean; + = infested; ? = inconclusive infestation; *d* = plant failed to grow; ( ) = plant died by end of experiment.

Tigridia. On plants infested with *Ditylenchus dipsaci* the lower parts of the leaf blades showed dark streaks, from which *D. dipsaci* was dissected, especially where the leaf sheath was thickened just above soil-level. The lesions were also present below soil-level, and there were pale flecks on the lower parts of the leaf blades. Symptoms shown by the plants attacked by *D. destructor* were similar to those caused by *D. dipsaci*, though there were no light flecks and the dark streaks were confined to the parts of the leaf sheath below ground-level. In these lesions and in one at the base of a bulb were numerous *D. destructor*. There were lesions on some

of the roots of these plants, similar to those on roots of bulbous iris caused by *D. destructor*, though here there were no eelworms present; the roots of plants attacked by *D. dipsaci* were quite healthy. Later, when the leaves had died, it was found that two bulbs from the *D. dipsaci* inoculation had died. A few pre-adult larvae of *D. dipsaci* were recovered from the dried leaves by extraction in a Baermann funnel. Four of the bulbs inoculated with *D. destructor* died and a number of *D. destructor* were obtained from the remaining bulbs.

Begonia. The control tubers remained healthy throughout, and increased in size both in 1949 and 1950. Strong symptoms of attack were shown by the plants in the pots inoculated with *Ditylenchus dipsaci* (Pl. 10, fig. 1). Two months after inoculation the early leaves exhibited small pimples, like spickles, but not differing in colour from the rest of the leaf; no eelworms, however, could be found associated with them. A month later these leaves had large discoloured areas which eventually shrivelled and, in one instance, the leaf dropped off. Part of a discoloured leaf was stained in Flemming's fixative after treatment in hot 80 % acetone. This revealed very many pre-adult *D. dipsaci* coiled up within the tissues. The stipules of the worst infested leaves were attacked; they, too, shrivelled and dropped off. The leaves and stem of *Begonia* are normally hirsute, but the leaves of infested plants bore very few hairs. The tubers were kept dry over winter and repotted, without any fresh inoculum, the following spring. Examination of the dry tubers had failed to reveal any eelworms, and the plants growing in 1950 also showed no symptoms of eelworm attack.

No symptoms were shown by the two plants inoculated with *Ditylenchus destructor*. One plant was very delayed in coming through the soil, but it grew and flowered as well as the other and the control plants. By the autumn, however, one tuber had rotted away and the other had suffered some damage. It was smaller than on planting and very irregular in shape, the irregularities being due to cavities containing dry rotted tissue. When this dead tissue was examined no eelworms could be found, neither could any lesions be seen on the tuber which had formed a new periderm underneath. This tuber was repotted in 1950, without any further inoculum, and by the autumn had become more normal in shape and did not show any rotted tissue. A second tuber, originally one of the 1949 controls, was potted in the spring of 1950, and the soil was inoculated with fresh *D. destructor* material. By the autumn there were a few lesions, one fairly large, where the stem had abscissed, but no eelworms could be found in them. In 1951 two fresh tubers were potted with a fresh inoculum of *D. destructor*. In the autumn, before the leaves had died down, the tubers were examined. As in 1949 the tubers were smaller than on planting and had irregular cavities containing rotted tissue; in one case the original skin of the tuber was still in position covering this tissue. No *D. destructor* could be found associated with the tubers. It seems probable that *D. destructor* causes damage to the tubers and possibly allows fungi to enter as well, but that usually the tuber is able to isolate the damage by the formation of a new periderm.

Gladiolus. Only plants in pots inoculated with *Ditylenchus dipsaci* showed any symptoms of attack, the rest looked quite normal. Infestation caused the leaves to twist and grow unevenly so that they were stunted to about half normal height (Pl. 10, fig. 2). Brownish lesions occurred on the sheathing leaf bases for a few inches above and just below soil-level. No spickles or puffy swellings were present on the infested leaves but they were paler green than normal. Many *D. dipsaci* were associated with the lesions on the lower parts of the leaves. All the gladioli flowered, but the flowers of the plants attacked by *D. dipsaci* were rather poor. By the end of August there were green seed pods, some of which were deformed and had lesions. In the bases of some of these pods several *D. dipsaci*, almost all pre-adult larvae, were found. When the leaves had died down they were removed and the corms examined. No lesions were found on any of the corms, and the following year the plants growing from them were free from disease. The dry leaves were broken up and put on a 'Seinhorst Mistifier' (Seinhorst, 1950; T. Goodey, 1951) when many pre-adult larvae of *D. dipsaci* were extracted from them. Similar treatment of the leaf bases of the plants inoculated with *D. destructor* yielded this species also, but in all stages. There were no lesions apparent on the leaf bases and these gladioli seemed not adversely affected in any way by the tuber eelworm.

At the end of the first year the corms were stored in a laboratory cupboard where the conditions were warm and dry. After the second year's experiments the corms were stored in an outside shed where conditions were cool and moist. On examination the following year several of the corms showed extensive damage. Part of this damage was due to *Botrytis* rot, but in some corms *Ditylenchus dipsaci* was found and in others *D. destructor*. It was not possible, however, to distinguish between the fungal and the eelworm damage.

Tropaeolum polyphyllum

Hastings (1940) found *Ditylenchus dipsaci* in tubers of *Tropaeolum polyphyllum* imported from Holland. He succeeded in setting up infestations, to varying extents, in eleven out of twelve varieties of potatoes that he tested. It seems possible, from his description, that the nematode involved was *Ditylenchus destructor* since the attack appears to have been confined to the tubers.

In September 1950 five tubers of *Tropaeolum polyphyllum* were planted in pots, each of which was inoculated with *Ditylenchus destructor* from potato. The tubers were quite healthy before planting. Early in 1951 shoots were produced, but after a while they died back. Examination showed that in all cases the tubers had rotted badly. In three out of the five tubers, numerous *D. destructor* were found. They were larger than normal, the females being half as long again as those from potato. Eelworms were also present in lesions on the underground parts of the shoot. These lesions were similar to those, caused by *D. destructor*, found on the haulms of potato.

Stachys palustris

Plants of marsh woundwort, *Stachys palustris*, were collected in October 1949 from a potato field near Holbeach, Lincs, where the crop was very badly attacked by *Ditylenchus destructor*. One of the rhizomes had brown lesions in which tuber eelworm was living and reproducing. Clean rhizomes were potted in soil with an inoculum of infested potato material, and transfer was eventually effected to the marsh woundwort under experimental conditions. The lesions caused by tuber eelworms were like those on mint rhizomes but were not so numerous. Although diseased rhizomes of mint are common in Fenland potato fields infested with tuber eelworm, many pieces of *Stachys* rhizome were examined before one was found with lesions. Two other common rhizomatous weeds of Fenland potato fields, coltsfoot, *Tussilago farfara* L., and persicaria, *Polygonum persicaria* L., have frequently been examined by the writer but no infestation by tuber eelworm has been found. The former has also been grown under experimental conditions in pots inoculated with *Ditylenchus destructor* but has not become infested.

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EXPLANATION OF PLATE 10

Fig. 1. *Begonia tuberhybrida* attacked by *Ditylenchus dipsaci*. Lower leaf on right is infested and has partially shrivelled.

Fig. 2. *Gladiolus hybridus*—three plants attacked by *Ditylenchus dipsaci*.

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GOODEY—*Host ranges of* D. destructor *and* D. dipsaci

INVESTIGATIONS OF THE INHIBITORY ACTION OF *BACILLUS SUBTILIS* ON *FUSARIUM UDUM* BUTL., THE FUNGUS CAUSING WILT OF PIGEON-PEA (*CAJANUS CAJAN* (L.) MILLSP.)

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A suitable basic medium for the growth of *Bacillus subtilis*, particularly in relation to the production of an antibiotic inhibitory to *Fusarium udum*, is described. Organic nitrogen with carbohydrate and K_2HPO_4 is shown to be essential for the growth of *Bacillus subtilis*. Magnesium sulphate, manganese and iron are found to increase the production of the antibiotic. Effects of temperature, period of incubation, aeration, etc., on the production of the inhibitory substance are described. The active principle is adsorbed by soil and to a lesser degree by kaolin. The organic matter and the soluble salts in the soil do not appear to play an important role in the loss of activity of the filtrates during passage through a bed of soil.

Introduction

In the previous investigation (Vasudeva & Roy, 1950), it was suggested that low incidence of pigeon-pea wilt or suppression of activity of *Fusarium udum* in the soil could probably be explained on the basis of the inhibitory activity of the associated soil microflora. Two isolates of *Bacillus subtilis* from soil showed marked inhibitory action on *Fusarium udum* in artificial cultures. The factors affecting the production of the antibiotic by *Bacillus subtilis*, as also the factors affecting its activity, particularly under natural conditions, have since been studied and are reported in this paper.

The production of toxic substances by antagonistic organisms has been shown to be dependent upon the pH of the medium, temperature, amount of inoculum, aeration and age of the cultures by Machacek (1928), Stubbs, Feeney, Lewis, Feustel, Lightbody & Garibaldi (1947), Brian, Curtis & Hemming (1948), Stroke & Woodward (1943), Waksman (1937) and Slagg & Fellows (1947). According to Slagg & Fellows (1947), Feeney, Garibaldi & Humphreys (1948) and Feeney & Garibaldi (1948), specificity of nutrients is a limiting factor for the production of the antibiotic substance.

There is no evidence to show whether these inhibitory substances are accumulated in the soil to give a concentration effective against the invading fungi. Broadfoot (1933) emphasized that the antagonism of a soil saprophyte to a plant pathogen, as measured by growth on artificial media, is not a reliable measure of the actual control exerted upon the parasite in the soil. Daines (1937), Cordon & Haenseler (1939), Christensen & Davies (1940) and Katznelson (1942) have demonstrated that

the toxic substance produced by a particular antagonistic organism, when added to the soil, becomes ineffective or is rapidly destroyed. Waksman & Woodruff (1940), Katznelson (1942), Kenknight (1941), Newman & Norman (1943) and Weindling (1946) have stated that to establish an antagonist in the soil by inoculation is very difficult. On the other hand, Millard & Taylor (1927), Weindling (1932), Allen & Haenseler (1935), Greaney & Machacek (1935), Novogrudski (1936), Vanluijk (1938), Anwar (1949), and others, have been able to demonstrate either the establishment of the antagonistic organisms or the lethal action of their anti-fungal substances in the soil. A temporary effect of an antagonist in soil has been observed by Weindling & Fawcett (1936) and Cordon & Haenseler (1939).

Material and method

The experimental work was conducted with the 'A' isolate of *B. subtilis* previously used by Vasudeva & Roy. The tests for the production of the inhibitory principle were made by growing *B. subtilis* in liquid medium described in the text, inoculated with a 1-day-old culture. The flasks were incubated at 30° C. or room temperature (26–28° C.) for 3 or 7 days respectively. After incubation the cultures were filtered through Whatman's filter-paper no. 42. One c.c. of the filtrate, autoclaved at 10 lb. pressure for 10 min., was mixed with 10 c.c. of potato-dextrose agar and poured in Petri plates. In controls 1 c.c. of the uninoculated medium was added. The plates were then inoculated in triplicate with *Fusarium udum* and incubated at 30° C. The growth of *F. udum* was measured every day.

Experimental

Nutritional requirements of Bacillus subtilis *in relation to the production of the inhibitory principle*

The production of the inhibitory principle on potato-dextrose agar has already been described (Vasudeva & Roy, 1950). In addition, Brown's medium, beef-extract peptone-broth, Dox's medium soil extract,* soil extract with 2% dextrose, soil-sannhemp medium,† sannhemp extract,‡ and sannhemp extract with 2% dextrose were tested for the production of the active principle. All these media were found to be unsuitable for the purpose. A basic medium with the following composition was set up and the effect of certain trace elements, e.g. Mn, Fe and Zn, on the production of the inhibitory substance was tested by the usual method.

Basic medium

Dextrose	20 g.	$MgSO_4$	0·5 g.
Asparagin	2 g.	KCl	0·5 g.
K_2HPO_4	1 g.	Distilled water	1000 c.c.

* *Soil extract.* Boil 200 g. of soil in 1000 c.c. of water for half an hour and filter to get a clear straw-coloured liquid.

† *Soil-sannhemp medium.* 100 g. of soil, 8 g. of sannhemp material and 50 c.c. water.

‡ *Sannhemp extract.* Steam 250 g. of green sannhemp material in 500 c.c. of water. Make up to 1000 c.c. with water.

$FeSO_4$, $MnCl_2$ and $ZnCl_2$: 0·001 % were added to the basic medium individually and in various combinations. The filtrate thus obtained was tested for its activity by incorporating it in potato-dextrose agar in the ratio of 1 : 10. Growth records of *Fusarium udum* after 2 days were taken and the percentage inhibition calculated on the basis of the control.

TABLE 1. *Effect of trace elements on the production of inhibitory principle*

Trace elements added to basic medium	Diameter of *F. udum* colony (mm.)		Inhibition (%)
	Control without filtrate	With 10 % filtrate	
$MnCl_2 + ZnCl_2$	70·0	24·7	65·0
$MnCl_2 + FeSO_4$	70·0	14·5	79·3
$FeSO_4 + ZnCl_2$	70·0	50·0	28·5
$FeSO_4 + ZnCl_2 + MnCl_2$	72·0	54·0	25·0
$MnCl_2$	71·5	24·5	65·5
$ZnCl_2$	65·0	46·0	29·0
$FeSO_4$	68·0	24·5	64·0
Basic medium alone	71·0	55·0	22·5

The results indicate that Mn and Fe are chiefly responsible for the increased production of inhibitory substance. Best results were obtained when both were present in the medium. Zinc chloride, when added with $FeSO_4$, appeared to have an adverse effect on the production of the active principle.

The medium with all the constituents and the trace elements Mn and Fe was compared with media in which the individual constituents were omitted. The data presented in Table 2 show the diameter of a 2-day-old *F. udum* colony with or without 10% filtrate.

TABLE 2. *Effect of individual constituents on the production of the active principle*

Medium	Diameter of *F. udum* colony (mm.)		Inhibition (%)
	Control (without filtrate)	With 10 % filtrate	
Complete basic medium with Mn and Fe	62·5	22·2	64·5
The above, less dextrose	.	.	Bacterium did not grow
The above, less asparagin	.	.	Bacterium did not grow
The above, less K_2HPO_4	.	.	Bacterium did not grow
The above, less $MgSO_4$	62·5	53·0	15·0
The above, less KCl	63·0	22·6	61·1
The above, less $FeSO_4$	63·0	32·0	49·2
The above, less $MnCl_2$	63·0	28·6	54·6

It is observed that media lacking in dextrose, asparagin and K_2HPO_4 are unsuitable for the growth of the bacterium. The importance of KCl, $FeSO_4$ and

$MnCl_2$ in the production of the inhibitory principle is clearly indicated, whereas $MgSO_4$ does not appear to be necessary.

Another experiment to test the effect of different concentrations of individual constituents was conducted, and comparative data are set out in Table 3.

Table 3. *Effect of different concentrations of individual constituents on the production of the active principle*

(All figures are percentages.)

Dextrose		Asparagin		K_2HPO_4		$FeSO_4$		$MnCl_2$	
Concentration	Inhibition	Concentration	Inhibition	Concentration	Inhibition	Concentration	Inhibition	Concentration	Inhibition
0·5	4·0	0·1	64·1	0·05	78·4	0·0001	33·0	0·0001	78·6
1·0	44·4	0·2	76·1	0·10	78·3	0·001	50·0	0·001	75·6
1·5	50·8	0·5	74·0	0·15	75·2	0·01	70·4	0·01	78·6
2·0	61·0	0·75	54·6	0·2	75·0	.	.	.	.
2·5	56·3	1·00	50·0	0·3	75·8	.	.	.	.
3·0	56·3	.	.	.	.	.	.	.	.
3·5	57·0	.	.	.	.	.	.	.	.
4·0	56·3	.	.	.	.	.	.	.	.
4·5	54·6	.	.	.	.	.	.	.	.
5·0	50·3	.	.	.	.	.	.	.	.

It was observed that appreciable inhibitory activity is produced in the medium over a range of concentrations of dextrose, asparagin, K_2HPO_4, $MnCl_2$ and $FeSO_4$.

The effects of different sources of carbohydrates on the production of inhibitory principle were tested, and the results are given in Table 4.

Table 4. *Different sources of carbohydrates as influencing the production of active principle*

Carbohydrates in the complete basic medium 2 %	*F. udum* (2-day-old) growth on P.D.A. (mm.)		
	Control	Filtrate of *Bacillus subtilis* 10 %	Inhibition (%)
Dextrose	53	17·5	67·0
Sucrose	53	16·8	68·3
Maltose	53	27·2	46·8
Gurh (sugarcane jaggery)	53	19·6	63·0
Lactose	53	53·3	0·0
Starch	53	24·7	53·4
Cellulose	56	56·3	0·0

The bacterium did not grow in the medium with lactose and cellulose. The maximum inhibitory principle was produced in the medium with dextrose, sucrose or 'gurh' for carbohydrates.

As sources of nitrogen both asparagin and peptone were tested and the inhibition percentage was 35·6 and 21·2 respectively. The trace elements, manganese chloride

and manganese sulphate, ferric chloride and ferric sulphate were tested. The inhibition percentage in all the four cases varied from 75 to 78.

In the light of the foregoing experiments dextrose-asparagin-phosphate medium, with the following composition, was chosen for the production of the inhibitory principle:

Dextrose or sucrose	20 g.
Di-hypophosphate	2 g.
Magnesium sulphate	0·5 g.
Potassium chloride	0·5 g. (not essential)
Manganese chloride	0·01 g.
Ferric sulphate	0·01 g.
Distilled water	1000 c.c.

The production of inhibitory principle is much more in dextrose-asparagin-phosphate solution than that in potato-dextrose solution. The comparative figures showing 3 days' growth of *F. udum* are given in Table 5.

TABLE 5

Medium used for growing *Bacillus subtilis*	*F. udum* growth on P.D.A. (mm.) Control without filtrate	Filtrate 10%	Inhibition (%)
Potato-dextrose solution	78	68	13
Dextrose-asparagin-phosphate solution	81	31	61·7

Effect of certain factors on the production of inhibitory principle

Temperature. The cultures of *Bacillus subtilis* on dextrose-asparagin-phosphate solution were incubated at 20–22, 24–26, 28–30, 32–34 and 37–40° C. for 4 days. The filtrates were assayed in the usual manner, and the results are given in Table 6.

TABLE 6. *Effect of temperature on the production of the inhibitory principle*

Incubation temperature (° C.)	*F. udum* (3 days' growth in mm.)	Inhibition (%)
Control (without filtrate)	76·3	.
20–22	67·0	12·2
24–26	27·0	64·6
28–30	27·7	63·7
32–34	28·3	62·9
37–40	34·8	54·3

The temperature range for the optimum production of inhibitory substance lies between 24 and 34° C., whereas at both higher and lower temperatures a fall in the active principle is indicated.

Period of incubation. The cultures of *B. subtilis* on dextrose-asparagin-phosphate solution were incubated at 28–30° C. from 3 to 21 days. The data showing the percentage of inhibition in relation to the period of incubation are presented in Table 7.

Table 7. *The effect of period of incubation on the production of inhibitory principle*

Period of incubation (days)	*F. udum* (3 days' growth in mm.)	Inhibition (%)
Control (without filtrate)	80	.
3	22·5	72·0
6	22·0	72·5
9	20·0	75·0
12	21·5	73·0
15	25·3	68·5
18	27·0	66·25
21	27·0	66·25

The data show that the inhibitory principle is more active up to 12 days' incubation, and that the activity after that period tends to decline.

Aeration. A slow stream of air sterilized by passing through concentrated sulphuric acid and then through freshly sterile medium to arrest sulphur dioxide was admitted into cultures of *B. subtilis*. The comparative activity of filtrates of aerated and unaerated cultures is given in Table 8. The maximum production of inhibitory principle in dextrose-asparagin-phosphate medium was observed at a range of pH between 6·5 and 8·5. Exposures to continuous light and darkness were found to be ineffective.

Table 8

Culture	*F. udum* (2 days' growth in mm.)	Inhibition (%)
Control (without filtrate)	48·7	.
(*a*) Aerated	37·5	25·2
(*b*) Un-aerated	22·2	48·2

Effect of Bacillus subtilis *or its cultural filtrate on* Fusarium udum *in soil*

Studies were made in 12 × 1 in. glass columns open at both ends and filled with: (*a*) soil-maize (crudely ground) (95:5), (*b*) sand-maize (95:5), and (*c*) soil-sand-maize (50:50:5). Initial moisture content was kept at 30%. The columns wrapped in paper were sterilized at 30 lb. for 1½ hr. These were then inoculated with 10 mm. agar culture of *Fusarium udum* at one end and *Bacillus subtilis* or 10 c.c. of its cultural filtrate at the other. The columns thus inoculated were kept at room temperature (25–34° C.). Linear growth of *Fusarium udum* was measured daily. The data are given in Table 9.

The data show that the growth of *F. udum* is not stopped in soil-maize and sand-soil-maize in the presence of *Bacillus subtilis* but the growth is retarded. In sand-maize, however, the growth seems to be checked after 13 days. The inhibitory effect of the filtrate appears to be more stable in sand-maize.

In another experiment air-dry sand and sand-soil (50:50) were impregnated with the cultural filtrate to 30% capacity. In the control the basic medium was used in

place of the filtrate. It was observed that the growth of *Fusarium udum*, which was very slow as compared to the controls to start with, was completely stopped after 5 days, i.e. fell to zero in sand impregnated with filtrate, whereas in sand-soil the growth was slow to begin with but almost normal in later stages.

TABLE 9. *Growth of* Fusarium udum *in soil columns in presence of* Bacillus subtilis *or its cultural filtrate*

Medium in columns	Organism introduced	Days: Growth of *F. udum* (mm.)															
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Soil-maize	*F. udum* alone	11	20	30	38	48	59	68	77	91	105	121	138	148	153	.	.
	F. udum vs. *B. subtilis*	12	20	25	33	40	49	62	70	78	82	90	98	104	111	117	123
	F. udum vs. filtrate	11	.	18	27	30	30	35	48	56	60	75	90	103	119	135	150
Sand-maize	*F. udum* alone	25	35	48	57	67	77	83	93	99	105	115	127	137	.	.	.
	F. udum vs. *B. subtilis*	25	33	45	51	62	73	77	82	86	88	90	90	.	.	.	.
	F. udum vs. filtrate	22	28	32	40	48	55	60	67	72	72	72	.	.	.	.	.
Sand-soil-maize	*F. udum* alone	15	23	35	48	58	68	80	95	100	109	120	.	.	.	.	.
	F. udum vs. *B. subtilis*	15	23	30	45	55	60	70	82	90	93	98	.	.	.	.	.
	F. udum vs. filtrate	15	22	33	48	58	70	83	95	100	110	120	.	.	.	.	.

Relative frequency of Fusarium udum *in soil in the presence of* Bacillus subtilis *and its cultural filtrate*

The experiment was set up in 6 in. earthenware pots which had been previously sterilized. These were divided into two series, the first containing the sterilized material and the other series containing unsterilized material. In each series pots contained soil alone, soil + 5% maize, and soil + 25% farmyard manure. One pot under each treatment in both the series was inoculated with 20 c.c. of spore suspension of *Fusarium udum*, the second pot with 20 c.c. of spore suspension of *F. udum* + 20 c.c. of *Bacillus subtilis* and the third pot with 20 c.c. of spore suspension of *Fusarium udum* + 20 c.c. of *Bacillus subtilis* filtrate. Clean slides were inserted into each pot up to half of their length, 7 days after inoculation. The slides were gently removed at intervals, fixed by flaming, stained with crystal violet and counter-stained with safranin. The slides were then examined for the presence of *Fusarium udum* spores. The relative frequency of *F. udum* spores on the slides thus exposed is shown in Table 10.

The results indicate that *F. udum* appears to persist in the sterilized soil in the presence of *Bacillus subtilis* and its filtrate as well as in controls. In the unsterilized soil, however, *Fusarium udum* was observed more frequently only in the first week, and suppression after 14 and 21 days is probably due to the other microflora present.

Table 10. *Relative frequency of* Fusarium udum *spores in the presence of* Bacillus subtilis *or its cultural filtrate*

Intervals in days when slides removed	Sterilized soil									Unsterilized soil								
	7			14			21			7			14			21		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Control (*F. udum* only)	+	++	+	++	++	++	++	+++	+++	+	+	+	−	+	+	−	−	−
F. udum–B. subtilis	−	+	+	+	++	++	++	+++	+++	+	+	−	−	−	−	−	−	−
F. udum–filtrate *B. subtilis*	+	++	++	++	++	++	++	+++	+++	+	+	+	−	−	.	−	−	−

A = soil; B = soil + 5 % maize; C = soil + 25 % farmyard manure;
+, *F. udum* spores present; −, *F. udum* spores absent.

Activity of cultural filtrate as influenced by soil

100 c.c. of culture of *B. subtilis* after 4 days' growth at 30° C. was passed through 1·5 cm. thick layer of soil through which water had been previously filtered. The filtrate was then assayed by the usual method and the results are given in Table 11.

Table 11

	Average diameter of *F. udum*, 3 days' growth (mm.)	Inhibition (%)
Control	81	.
Normal filtrate	31	61·7
Filtrate passed through soil	75	7·4

It appeared that the filtrate during its passage through the bed of soil appreciably lost the active principle. It was, therefore, considered necessary to determine if soluble salts in soil have any effect on the activity of the filtrate. For this purpose, soil-extract agar was prepared by adding 2% agar to soil extract.* One c.c. of the autoclaved filtrate was added to 10 c.c. of soil-extract agar and poured in Petri plates. These were then inoculated with *Fusarium udum* and incubated at 30° C. It was observed that soluble salts present in soil extract do not adversely affect the activity of the filtrate. The data are given in Table 12:

Table 12

	Average diameter of *F. udum*, 3 days' growth (mm.)	Inhibition (%)
Control (soil-extract agar), 10% basic medium	65	.
Soil-extract agar, 10% filtrate	31	52·3
Control: potato-dextrose agar, 10% basic medium	84	.
Potato-dextrose agar, 10% filtrate	40	52·4

Tests were also carried out to determine the possible effect of organic matter in the soil on the activity of the filtrate. For this purpose cultures were passed through

* Soil extract prepared by boiling 200 g. of soil in 1000 c.c. of water for half an hour. After filtration, the clear straw-coloured liquid was made up to 1000 c.c.

a layer of soil previously ignited at 500–600° C. The filtrate was then assayed and the results are given in Table 13.

TABLE 13

	Average diameter of *F. udum*, 3 days' growth (mm.)	Inhibition (%)
Control	80	.
Normal filtrate	34	57·5
Filtrate through soil	78	2·5
Filtrate through ignited soil	77	3·75

There appears to be no appreciable difference in the activity of the filtrates passed through a bed of soil and through soil which had been ignited.

The cultures were also filtered through 1·5 cm. thick layers of sand, soil and kaolin separately. The inhibitory activity of the filtrates thus obtained was assayed in the usual way. The data are set out in Table 14.

TABLE 14. *Inhibitory activity of the filtrate as influenced by sand, soil and kaolin*

	Average diameter of *F. udum*, 3 days' growth (mm.)	Inhibition (%)
Control	84	.
Normal filtrate	40	52·4
Filtrate through sand	45	46·4
Filtrate through soil	83	1·2
Filtrate through kaolin	76	9·5

The data show that the filtrates during passage through soil and kaolin lose their activity appreciably, whereas the loss of activity during passage through sand is not marked.

During these experiments it was observed that the pH of the cultures after passage through beds of soil and kaolin shifts from 5·7 to 7 approximately. Adjustment of pH to its original status did not restore the activity of the filtrates.

The soil or kaolin after filtration of the cultures, when incorporated in potato-dextrose agar had no adverse effect on the growth of *Fusarium udum*.

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FACTORS AFFECTING THE SURVIVAL OF *PLASMODIOPHORA BRASSICAE* WOR. IN THE SOIL AND ITS ASSESSMENT BY A HOST TEST

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(With 3 Text-figures)

The activity of *Plasmodiophora brassicae* Wor. in the soil was measured simultaneously by counts of root-hair infection on cabbage seedlings and percentage clubbing of older plants. A linear relation was found between the logarithms of the mean number of root-hair infections per seedling and the logarithms of the spore concentration in the inoculum. It was found that a high proportion of clubbed plants could be associated with very low average numbers of root-hair infections on seedlings. The percentage of clubbed plants can be used for assessing quantitatively the infestation of soil by *P. brassicae* but only at lower levels of infestation than can counts of root-hair infections. An effect of early application and higher levels of nutrients in increasing clubroot indicates that the nutrient status of the soil is also important, but it is not clear whether this is due to increased club growth or to a direct effect on the fungus.

Experiments on the survival of resting spores showed that, in a wet acid soil, there was a rapid decline in numbers of infections to a low and nearly constant level with increasing time from inoculation. This decline could be considerably delayed by alkaline or dry soil conditions or by the addition to the soil of mustard oil (allyl isothiocyanate). This fall in activity is ascribed to spontaneous germination of resting spores, germination being prevented or delayed by such conditions as alkalinity, low soil moisture or the presence of inhibitors.

In an attempt to cause biological stimulation of resting-spore germination by growing short-term catch crops in infested soil (re-infestation of the soil from diseased crucifers being prevented), there were fewer infections on test cabbage seedlings after crucifers or ryegrass had been grown than after a number of other non-crucifers. In fallow soil, a similar reduction was, however, caused by adding nutrients alone.

It was confirmed that *P. brassicae* can infect the root hairs of a number of non-crucifers and form zoosporangia and zoospores, but no other stages of the fungus have been seen in those plants.

Possible improvement of the sampling design in experiments using the root-hair infection-count method is briefly discussed.

The prolonged persistence in the soil of *Plasmodiophora brassicae* Wor., the cause of clubroot of crucifers, is one of the chief obstacles to its eradication. Fedorintschik (1935), Gibbs (1939), Nielsen (1933), and others, have recorded outbreaks of the disease in the soil for periods up to 7 years after a susceptible crop. This has generally been attributed to the survival of resting spores which are produced in enormous numbers in infected plants. Proof of resting-spore survival, however, is lacking, because most conclusions on longevity are based on field observations and

the possibility of the fungus maintaining itself on cruciferous weeds has not always been excluded. Gibbs's observation (1939) of diminishing soil infectivity with time is, nevertheless, consonant with a diminishing number of surviving resting spores. A hitherto unsuspected method of survival is suggested by Webb's (1949) discovery of the zoosporangia in some non-crucifers, including several grasses. Although resting spores may not provide the only method of survival they must be important for the persistence of the fungus.

Environmental factors controlling infection and hence presumably resting-spore germination have been studied by many investigators. In general, the results indicate that a pH of about 6, high soil moisture, a temperature of 25° C. and good aeration are the conditions optimal for infection. By counting full and empty spores from pieces of clubroot gall buried in the soil, Bremer (1924) recorded that up to 37% spores had germinated after 11 days in a wet acid soil, whereas in an alkaline soil only 1–2% had done so. Bremer (1924) stated that soil conditions favouring spore germination would, in the absence of host plants, be likely to favour rapid disappearance of the fungus. Chemical stimulation of resting-spore germination by mustard oils, commonly present in crucifers, has been claimed by Hooker, Walker & Link (1945). Noble (1924), working with *Urocystis tritici*, recorded a similar effect with benzaldehyde as well as by fragments of host tissue.

Biological stimulation of spore germination by the host is a well-known phenomenon (Brown, 1946). Some plants that are not susceptible also produce the necessary stimulus, and the use of such plants as decoy crops has been attempted.

The experiments to be described below were designed to investigate the effect on spore survival of varying environmental factors such as pH and moisture content, and of possible means of stimulating germination.

Methods available for assessing spore survival include finding the percentage clubbing of test plants and the method developed by Samuel & Garrett (1945) whereby root-hair infections are counted. The technique of counting root-hair infections in young seedlings has the advantage of permitting better control of environment during the test; it also gives more quantitative results and was therefore used extensively in the present work. In contrast to a direct method of counting germinated resting spores, such as used by Bremer, this method measures only those spores which have both germinated and succeeded in establishing an infection. The method clearly can be applied only during the period of susceptibility of the part of the root on which a count is made. No such simple relationship with germination can be postulated for a count of clubbed plants, because the life cycle of *Plasmodiophora brassicae* is incompletely known, but the production of clubroot symptoms is the practical criterion of activity and survival of the fungus. As a preliminary to interpreting the results obtained by these two methods it was desirable to compare them under identical conditions, and experiments were made to relate the proportions of clubbed plants and the number of root-hair infections on seedlings, at varying spore concentrations.

Methods

Soils were prepared according to the methods described by Garrett (1938). Rothamsted allotment soil was acidified with sulphuric acid to bring the reaction to approximately pH 5·8. When alkaline conditions were required soil was treated with 0·25% by weight of $Ca(OH)_2$. All soils were diluted with three times their volume of sand. The saturation capacity of these soil-sand mixtures, determined by the Keen-Raczkowski (1921) method, varied very little from 24. Determinations of pH were made with a quinhydrone electrode. When seedlings were grown for root-hair infection counts only, they were raised in soil contained in glass tumblers of 250 ml. capacity. For experiments involving club formation or where crop treatments were applied, boxes measuring $14 \times 9 \times 3$ in. were used. When nutrients were applied, they were given as $NaNO_3$, $(NH_4)_2SO_4$ and KH_2PO_4.

Clubroot galls were sometimes collected in the field from naturally infected plants but were usually produced in the glasshouse. For some experiments inoculum was stored at $-8°$ C. but under such conditions it became non-viable in 6 months.

Spore suspensions were prepared by macerating the galls, and their density determined by haemocytometer count. For soil-moisture contents of 80% saturation, inoculum together with any soluble substances under test was diluted in the whole volume of water and added to the surface of the air-dry soil. When the desired moisture content was 30% saturation or when boxes were being used, soil and spore suspension were mixed and then filled into the containers. In survival experiments of long duration water contents were maintained by watering twice weekly.

Cabbage plants for clubroot counts were grown for about 6 weeks from the time of sowing, in a warm greenhouse. About a hundred seedlings were raised in each box, some of which were used for root-hair infection counts leaving about fifty for the final assessment of clubbing. The plants were sorted into the following categories depending on the severity of the symptoms:

(1) Dead and decaying
(2) Severe tap-root clubs } total severe tap-root clubs.
(3) Slight clubroot.
(4) No symptoms.

For the root-hair infection-count method, samples were taken at various times, usually beginning 24 hr. after inoculation. Twenty germinating cabbage seeds were sown in each tumbler flush with soil-level and covered with a thin layer of sand. Seedlings were grown at 25° C. in the dark for 1 week and washed out at this time. The roots were stained and preserved in 0·1% aceto-carmine. When counts were made on seedlings grown in boxes, groups of four adjacent seedlings were picked at random one from each of the five rows of plants in the box. They were removed carefully together with the soil round the roots, washed, and stained

Ayers (1944) has indicated that each zoosporangial plasmodium is derived from a separate zoospore infection and that if several zoospores penetrate one cell the plasmodia remain distinct. Hence the number of separate zoosporangial groups was counted rather than the number of infected root hairs. Counts were confined to clearly differentiated sporangia since earlier stages could not always be recognized with certainty. Development of zoosporangia on a given segment of root is usually fairly uniform and there were few doubtful infections. Counting was simplified by mounting the root on a slide ruled at convenient intervals (usually 1 mm.). Infections counted were usually those on the oldest 3 cm. of root or on the 2nd and 3rd cm. Variations in this practice are indicated in the tables. Most root hairs were spread out on either side of the root, and when infections were numerous the error introduced by ignoring infections lying on or below the root itself was small (Samuel & Garrett, 1945). When infections were few and the omission of any would be important, an effort was made to include every infection on the segment of root examined.

The standard error of root-hair infection counts was found to vary with their mean so that error distribution of original values was not normal. The transformation log $(x+1)$, where x was the number of infections, was found to correct error dependence on the mean. The transformed figures were subjected to analysis of variance. For the analysis of percentage clubroot figures, the angular transformation was used. Where possible, original values have been given in the tables beside the transformed figures.

RESULTS

Inoculum and intensity of infection

The effect of varying the spore concentration on the intensity of infection was studied in four experiments. Table 1 gives the details of these experiments relevant to the root-hair infection counts. Spores of different degrees of maturity were also contrasted in Exps. 2 and 3, with newly formed spores from firm white galls and spores from decaying tissues respectively. The nutrient treatments applied in Exps. 2 and 3, and described below, had no effect on the number of root-hair infections. Table 2 shows the counts of root-hair infections.

Actual numbers of infections increase rapidly with increase in spore concentration, the rate of increase tending to fall at higher infection densities. The log of the mean number of infections per root was plotted against log spore concentration and a linear relationship found. Regression lines were calculated and are shown in Fig. 1.* The low values, however, cannot be regarded as determined with precision. It is found that the curves differ little in slope from experiment to experiment, which probably reflects the similar relation between numbers of swimming zoospores and numbers of potential infection sites on the root. The slope indicates

* To obtain the figures in Table 2, counts on individual seedlings were increased by one and logarithms taken. The points in Fig. 1 are based on transformed *mean* values, and therefore do not correspond directly with the figures tabulated.

approximately that a threefold increase in infection numbers requires a sevenfold increase in spore concentration. The greater number of young spores required to produce a given level of infection is probably due to a much smaller proportion of them being ready to germinate and infect the host cell.

TABLE 1. *Details of experiments on spore concentration and root-hair infection*

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
No. of spore concentrations	5	4	4*	4
Range of spore concentration/ml. inoculum	10^3 to 64×10^3	10^3 to 10^6	10^3 to 10^6	10 to 10^4
Factor of increase in spore density at each step	(×8)	(×10)	(×10)	(×10)
Spores	Mature	Young	Mature	Mature
Experimental conditions	Dark incubator, 25° C.	Glasshouse, summer. Carried out simultaneously		Glasshouse, summer
No. tumblers or boxes for each spore concentration	4†	4	8	4
No. of roots examined per tumbler or box	5	10	10	10

* There was another series with 10^7 spores/ml. but infections were not counted. See Table 4.
† Tumblers

TABLE 2. *Numbers of root-hair infections at different spore concentrations*

(Numbers of infections/2 or 3 cm. root. Means of transformed values.)

Spore concentration/ml. inoculum	Exp. 1 (2 cm.), mature spores	Exp. 2 (3 cm.), young spores	Exp. 3 (3 cm.), mature spores	Exp. 4 (3 cm.), mature spores
10	—	—	—	0·02
100	—	—	—	0·09
1,000	0·153	0·08	0·31	0·36
8,000	0·714	—	—	—
10,000	—	0·17	0·91	1·22
64,000	1·194	—	—	—
100,000	—	0·68	1·94	—
512,000	1·961	—	—	—
1,000,000	—	1·34	2·65	—
4,096,000	2·253	—	—	—
Significant difference ($P = 0·05$)	0·279	0·130	0·151	0·127

Percentage clubbing in relation to treatments was also assessed in three of the above experiments (see Table 3). Results are presented as the percentage of plants with severe tap-root clubs in Table 4. Percentage clubbing also increases rapidly with rising spore concentration, the rate declining more as higher proportions of plants become infected. Somewhat similar results have been described by Gibbs (1931). As with root-hair infections, mature spores were more effective than young spores in causing clubroot. In Exp. 4 there was much more clubroot at the lower spore concentrations used than might have been expected from the results of Exp. 3. This can probably be accounted for by the striking effect of the nutrient treatments on the proportion of diseased plants. Early application in particular, as well as the

higher level of nutrients, favours development of clubs. The treatments are relatively ineffective at high spore concentrations, presumably because a very high

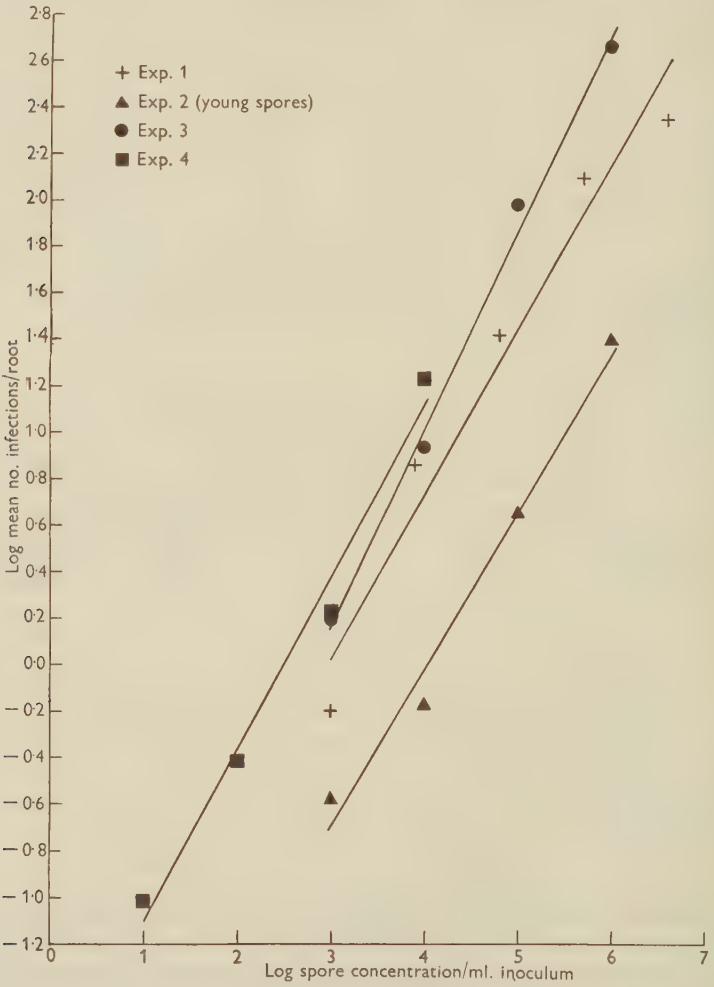

Fig. 1. Regression of log mean no. infections per root on log spore concentration in the inoculum.

proportion of the plants will be infected at all nutrient levels. There is also an interaction of nutrient level with time of application, higher doses of nutrients increasing clubroot only when applied early. Thus the figures for the higher-level-early application series in Exp. 3 correspond much more closely with those for

similar spore loads in Exp. 4, which received this nutrient treatment throughout, than do the general means for each level of inoculum.

TABLE 3. *Details of experiments on development of clubroot*

Exp.	No. replicate boxes	No. spore concentrations	No. nutrient treatments	Sowing date	Weight each salt applied		Times of application		Harvest date
					Low	High	Early	Late	
2	2	4	2	15. vi	0·23	0·93	18. vi	—	—
					0·23	0·93	9. vii	—	31. vii
3	2	5	4	15. vi	0·23	0·93	18. vi	9 vii.	—
					0·23	0·93	9. vii	26 vii.	3. viii
4	4	4	1	29. vii	—	1·86	3. viii	—	8. ix

TABLE 4. *Percentage of plants with severe tap-root clubs at various spore concentrations and with different nutrient treatments*

Exp. 2. Means of transformed values (original figures in brackets)

Time of application	Nutrient level	Spore concentration/ml. inoculum			
		10^3	10^4	10^5	10^6
Early	Low	11·4 (4)	37·4 (37)	58·3 (70)	78·5 (96)
	High	19·6 (12)	52·0 (62)	76·0 (94)	77·4 (94·7)

Significant difference = 18·6 ($P = 0·05$)

Exp. 3. Means of transformed values (original figures in brackets)

Time of application	Nutrient level	Spore concentration/ml. inoculum				
		10^3	10^4	10^5	10^6	10^7
Early	Low	34·4 (32)	64·0 (79)	76·0 (94)	90·0 (100)	90·0 (100)
	High	52·0 (62)	78·2 (92)	90·0 (100)	90·0 (100)	86·0 (99)
Late	Low	23·8 (17)	42·2 (45)	77·2 (95)	81·9 (98)	90·0 (100)
	High	18·3 (11)	46·2 (52)	68·0 (86)	81·9 (98)	90·0 (100)

Significant difference = 12·42 ($P = 0·05$)

Exp. 4. Means of original figures

Time of application	Nutrient level	Spore concentration/ml. inoculum			
		10	10^2	10^3	10^4
Early	High	0·5	29·7	86·5	99

To compare the root-hair infection counts with percentage clubbing in the three above experiments, Figs. 2 and 3 show percentage plants clubbed plotted against the number of root-hair infections on a logarithmic scale, the latter being chosen merely for convenience. Mean values for each spore concentration are given in Fig. 2, and values for the separate treatments in Exp. 3 in Fig. 3.

The effects of the nutrient treatments on clubroot as described above are shown in Fig. 3. Comparison of series receiving similar nutrient supply does, however,

show a fairly good correspondence between experiments in the number of diseased plants associated with any particular level of root-hair infection. The feature of these results, which is most emphasized by the graphs, is that a considerable proportion of plants may develop clubroot when the root-hair infection count records an average of less than one per plant. Thus, in Exps. 2 and 4 there was 50–60%

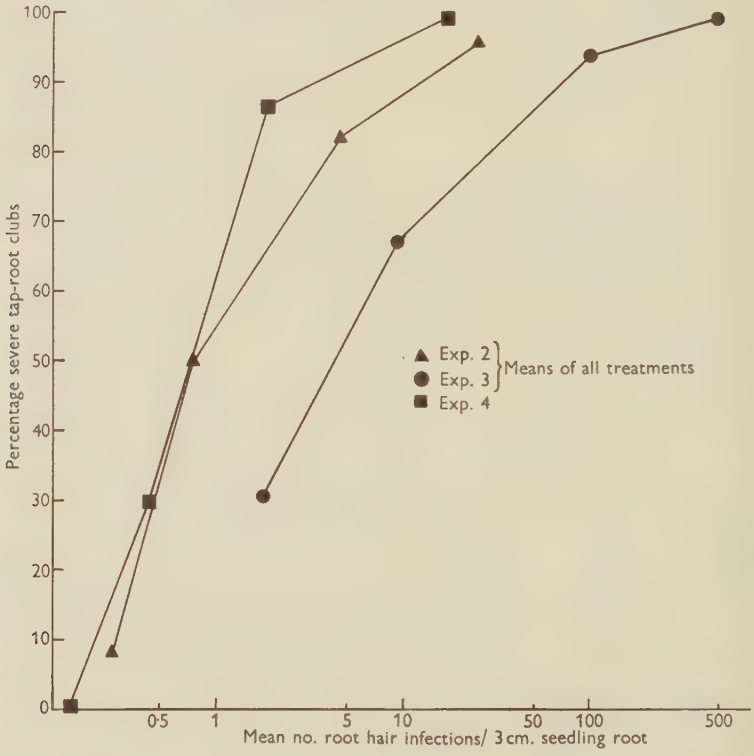

Fig. 2. Percentage severe tap-root clubs and corresponding mean root-hair infection counts on seedlings.

clubroot with an average root-hair infection count of 1 per plant, while there was 50% clubroot when the infection count was as low as 1 on every sixth plant. It would therefore appear that a clubbing test can give a satisfactory quantitative measurement of low ranges of activity, provided due attention is paid to such factors as nutrient supply, which may affect the development of clubs.

Spore concentrations as encountered in the field are presumably low, so that for ecological experiments simulating field conditions a clubbing test would probably be both adequate and appropriate. The greater time required to produce clubs

might well be more than offset by the avoidance of the tedious and time-consuming microscopic counts.

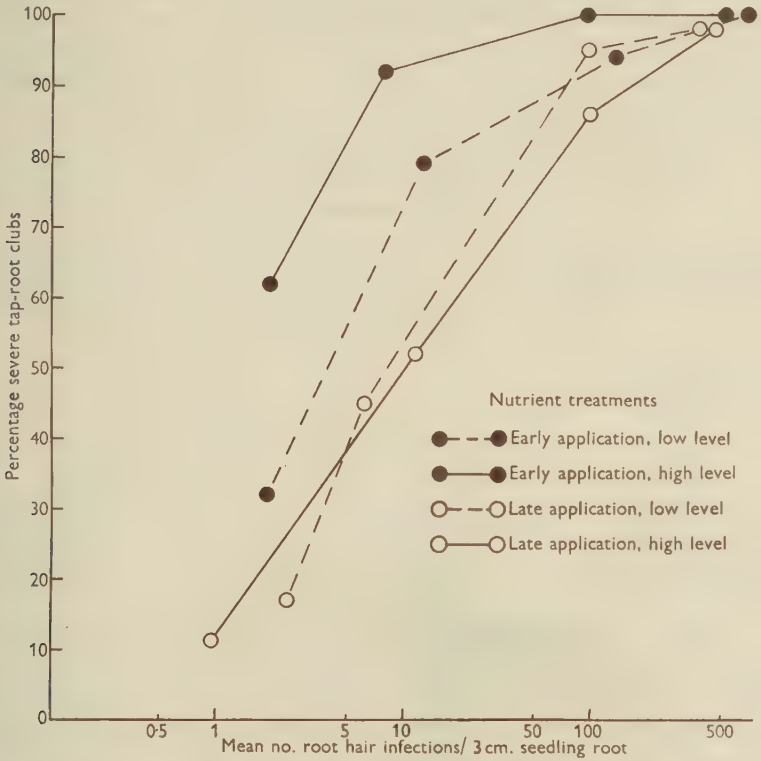

Fig. 3. Percentage severe tap-root clubs and corresponding mean root-hair infection counts on seedlings for the separate treatments in Exp. 3.

Experiments on survival

In view of Bremer's discovery of considerable spore germination in the soil, the activity of *P. brassicae* was followed at various times after inoculation to a soil favourable to infection. In Exp. 1 infectivity was tested at all five spore concentrations at seven sampling times. Counts are given in Table 5, which also shows pH values of the soils at the time of washing out seedlings. At each spore concentration there is a fall in activity to a more or less steady value, the rate of fall decreasing with time. There is a slight rise at the last sampling, which is just significant at the highest spore concentration. The rate of decrease at the three highest spore levels is nearly exponential during the initial period of decline, but such a relationship obviously does not hold over the whole period of sampling. At the low spore

concentrations, the decrease is less pronounced but in this range differences seldom exceed the standard error, and the transformation itself distorts these low values considerably. Of soil factors which might affect the numbers of infections, only pH was recorded at each sampling time and this changed little during the experiment. Such a decline in infectivity is to be expected, if spores germinate spontaneously, the rate decreasing with time as an increasing proportion of the remaining population would consist of the more dormant spores.

TABLE 5. *Root-hair infections at different spore concentrations and at different times after inoculation*

(Mean laboratory temperature 14° C. Soil moisture maintained at 80% saturation. Initial soil pH 5·8. Four replicate tumblers/treatment. Counts on 2 cm. root on 5 seedlings/tumbler.)

	Means of transformed values						
	Days after inoculation						
Spore concentration/ml. inoculum	1	5	10	16	22	29	99
1,000	0·153	0·283	0·069	0·000	0·030	0·030	0·078
8,000	0·714	0·745	0·084	0·252	0·045	0·075	0·168
64,000	1·194	1·010	0·888	0·642	0·444	0·456	0·530
512,000	1·961	1·420	1·457	1·294	0·836	0·884	0·839
4,096,000	2·253	2·364	1·847	1·629	1·386	1·104	1·532
	Significant difference = 0·279 (P = 0·05)						
Soil pH at time of washing out seedlings	6·04	5·99	6·17	6·40	6·06	6·26	6·37

Effect of pH. To test this hypothesis, an experiment was set up to obtain some information on survival under conditions both favourable and unfavourable to infection. Spores were added to acid soil (pH 5·8) and limed soil (pH 8·0) at two moisture levels, 80 and 30% saturation. Further details are given in Table 6. To test the activity of *P. brassicae* it was necessary first to bring all soils to a condition suitable for infection. Soil was removed from the tumblers and mixed with an equal volume of acid soil, the moisture content being maintained, where necessary, at 40% saturation. Tumblers were then filled and water added to 80% saturation; H_2SO_4 was also added to the alkaline soils in amounts equivalent to the lime originally added. Results in Table 6 are given as percentages of the mean value for each sampling time. Results for one sampling are thus not directly comparable with those for another. Table 6(*a*) shows that, after 6 weeks, activity has fallen to a lower level in the wet acid series than in the others. After 24 weeks, however, it has fallen to the same level in all soils save that most unfavourable for germination, namely, the dry, alkaline soil. This suggests that dry and alkaline conditions had delayed spontaneous germination. On acidification of the soil at sampling time all spores hitherto kept dormant would germinate, and more numerous infections result. This is supported by the results obtained when an interval of 1 week is interposed between acidification of the soil and sowing. Table 6(*b*) shows that the

infectivity of the dry alkaline soils is now as low as that of the acid soils. That acidification and incubation at 25° C. however may have effects other than that suggested here is indicated by the tendency for infections to be fewer in the wet-limed series than in the others, the difference being significant in the sample taken after 24 weeks.

TABLE 6. *Activity of* Plasmodiophora *after different times in acid and limed soils, each kept at two moisture levels*

(Mean laboratory temperature 16·3° C. Spore concentration 200,000 spores/ml. at 80 % saturation. Three tumblers/treatment, each divided for test, giving three pairs for each treatment. Counts on 2 cm. root on 5 seedlings/tumbler.)

		Percentage of means of transformed values for each sampling time				
Time of sampling. Weeks after inoculation	Treatment after acidification	Acid		Limed		Significant difference
		Wet (80 %)	Dry (30 %)	Wet (80 %)	Dry (30 %)	
(*a*) 6	Sown at once	74·15	111·8	102·6	111·5	13·6
30	Sown at once	91·47	99·08	92·45	117·0	14·35
(*b*) 24	Interval of 1 week	107·47	101·78	90·90	99·78	10·27
30	before planting, temp. 25° C.	99·08	111·84	92·21	96·94	23·09

Effect of allyl isothiocyanate. An effect somewhat similar to that of low soil moisture and alkalinity was obtained in an experiment in which different concentrations of allyl isothiocyanate (AI) were added to soil with the inoculum. The results are shown in Table 7, and again a large fall in activity has taken place in the

TABLE 7. *Root-hair infections at two times after treatment with different concentrations of allyl isothiocyanate*

(Spore concentration 250,000/ml. at 80% saturation. Mean laboratory temp. 15·7° C. Soil moisture kept at 80 % saturation. pH 5·7. Two replicate tumblers for 1st sample, three for 2nd. Control replication doubled. Counts on 2 cm. root on 5 seedlings from each tumbler.)

	Means of transformed values					
	Concentration of allyl isothiocyanate/ml. inoculum (p.p.m.)					
Time of sampling	0	5	10	20	40	80
Immediately after inoculation	2·95	2·71	2·41	1·46	No seedling growth	
	±0·095		±0·012			
6 weeks later	1·30	1·93	2·00	1·78	1·61	0·60
	±0·079			±0·011		

untreated soil during the 6 weeks after inoculation. At the initial sampling the concentrations of AI were so high in the 80 and 40 p.p.m. series that the seedlings were killed at an early stage. At lower concentrations there was reduction in infection compared with the controls, the effect becoming less with increasing dilution. After 6 weeks seedling growth occurred in all series, indicating that the concentration of AI was much reduced, having either diffused away or become decomposed or fixed

in the soil. There was slight infection in the 80 p.p.m. series, but it was still at a level lower than the control, indicating either destruction of the spores or inhibition of infection. At all other concentrations, however, infections were higher than in the control, increasing with diminishing concentration of AI from 40 to 10 p.p.m. when there was a slight reduction at 5 p.p.m. Compared with the first sampling, there were more infections at the 80, 40 and 20 p.p.m. levels, again probably indicating a reduction in the effective concentration of AI in the soil. At the 10 and 5 p.p.m. levels there were fewer infections than at the first sampling. If, as suggested above, the reduction in activity in untreated soil, which was very marked in this experiment, is due to previous spontaneous germination of spores, then it would appear that, in these two series, spontaneous germination has taken place but was delayed by a slightly inhibitory concentration of mustard oil. Essentially similar results to the above were obtained when benzaldehyde was used with the same concentration range.

Effect of previous crop. In view of the possibility that some plant roots might stimulate germination of resting spores, infected soil was cropped in various ways, and the subsequent activity of *P. brassicae* assessed by the root-hair infection-count method. Both susceptible and reputedly resistant crucifers were used, in addition to some non-crucifers, selected because of chemical or taxonomic affinities with the Cruciferae. Details of the two experiments in which these effects were explored are given in Table 8. Since it was necessary to supply nutrients to the plants, two fallow treatments were included in Exp. 7, one with and one without added salts.

TABLE 8. *Details of experiments with cropping treatments*

Exp.	Initial soil pH	Spore conc./ml. water at 80 % saturation	Date begun	Total wt. each nutrient salt added (g.)	Times of application of nutrients	Date of sampling	No. boxes per treatment	No. tumblers from each box
7	5·8	875,000	4. ii	1·86	8. iii, 30. iii, 25. iv	4. v	2	2
	5·8	875,000	11. x	0·7	19. x, 17. xi	23. iii	2	2

Cabbage and kale plants were removed as soon as symptoms of clubroot were apparent and fresh sowings made. *Lepidium* plants showed no signs of clubroot until towards the end of the experiment and the roots had not decayed when the plants were removed. Neither species of *Matthiola* developed clubroot. At sampling enough soil to fill at least two tumblers was removed from random positions in each box. The numbers of infections on the test seedlings and pH values at the time of washing out are given in Table 9. Infectivity has, as in other experiments, fallen much below its initial value. Addition of nutrient salts to the fallow has reduced infection numbers. Infections after crucifers are fewer than after untreated fallow, but only with cabbage 1 and *M. incana* are they significantly fewer than after fallow treated with nutrients. After the non-crucifers, infections were little different from the untreated fallow, and hence higher than after crucifers or

fallow with nutrients. The marked difference between numbers of infections after crucifers and after non-crucifers suggests an effect of the type which was being sought, and that the crucifers had lowered the number of viable spores in the soil by stimulating some of them to germinate. Analysis of covariance of root-hair infection counts and soil pH did not show any dependence of one on the other, so that differences can be attributed to effects of the treatments other than their influence on soil reaction. In this experiment, after removing the soil samples for infection tests, all the boxes were sown with cabbage. More than 70% of the plants in every box developed severe clubroot. As might have been expected with such high numbers of root-hair infections on the test seedlings, there were no significant differences between number of plants clubbed in the various soils.

The results of another such experiment are also given in Table 9. Infection numbers are fewer after *Lolium perenne* than after the others. There was a suggestion of a similar effect with carrot. The values for the other treatments did not differ significantly among themselves.

TABLE 9. *Root-hair infections on seedlings after various cropping treatments of infested soil*

(Counts on 2 cm. root on 5 seedlings/tumbler.)

Exp. 7

Treatment	Means of transformed values	pH of soils at time of washing out seedlings
Initial infectivity	2·778	—
Fallow	2·078	6·72
Fallow + nutrients	1·644	6·37
Cabbage 1	1·190	6·38
Cabbage 2	1·502	6·28
Kale	1·385	6·37
Lepidium sativum	1·579	6·28
Matthiola incana	1·066	6·60
M. bicornis	1·626	6·30
Tropaeolum majus	2·004	6·27
Bean	2·044	6·03
Onion	2·179	6·28

Significant difference = 0·36 ($P = 0·05$)

Exp. 8

Treatment	Means of transformed values
Fallow	1·745
Reseda odorata	1·715
Tropaeolum majus	1·660
Lolium perenne	1·164
Wheat	1·868
Tomato	1·832
Carrot	1·493

Significant difference = 0·41 ($P = 0·05$)

Observations on susceptibility to root-hair infection and clubroot

Examination of the root hairs of plants grown in the foregoing and other experiments confirmed Webb's discovery of zoosporangia, indistinguishable from those of *Plasmodiophora brassicae*, in non-cruciferous roots. Zoosporangia were seen in the root hairs of *Reseda odorata*, *Lolium perenne* and *Tropaeolum majus* grown in the crop-rotation experiments, but no infections were seen in the other plants. A small infection experiment with inoculated and uninoculated soil showed infection of the plants mentioned and also of *Papaver rhoeas*, *Agrostis alba stolonifera* and *Dactylis glomerata*. No zoosporangia were seen in the roots of wheat, oats, barley, rye or maize. The plants grown in uninoculated soil were free from such infections so that there seems to be little doubt that the zoosporangia seen were actually those of *Plasmodiophora brassicae*. No other recognizable stages of the fungus have so far been seen in those plants, and the fate of the zoospores is unknown.

Seedlings of *Matthiola incana* and *M. bicornis*, both species listed by Karling (1942) as highly resistant to clubroot, were found to have root-hair infections with zoosporangia, but at no time were symptoms of clubroot found nor were plasmodia or spores seen in the tissues. On the other hand, marrow stem kale, frequently reported to be relatively resistant to clubroot was, in Exp. 7, completely susceptible, 90% of the plants being severely affected. *Lepidium sativum* was employed in Exp. 7 because of its reported high resistance to clubroot (Karling, 1942). After 8 weeks or so, the hypocotyls of some of the plants began to swell slightly and examination of the roots showed symptoms of clubroot. At the end of the experiment, 90% of the plants had clubroot and spores were present in the infected tissues.

DISCUSSION

The most important result which has emerged from the survival experiments described above is the large and rapid fall in infectivity of the soil with increasing time from inoculation. This has been attributed to spontaneous germination of the resting spores. The decrease in the rate of fall would be expected as the proportion of more dormant spores gradually increased. Of course, the view might be advanced that the effect is due not to previous germination of spores but to a reduced capacity for germination when the host plants are sown. Other recent experiments with spore suspensions, however, not described here, involving direct spore counts have shown that they do germinate, fully confirming Bremer's work. The results of experiments with lime and with mustard oil may therefore be interpreted as direct effects on this spontaneous spore germination. The inhibition of such germination by liming is likely to be only temporary, as is evident from the experiment on survival in limed soils, and to this extent liming in the field may actually increase the persistence of the pathogen. Similar effects were occasioned by low soil moisture and inhibitory substances such as mustard oil. In the experiment with different concentrations of mustard oil, survival was not assessed over different periods but

only at one time. The rise in infection in the series from high to intermediate concentrations and the subsequent fall as the concentration is further reduced is entirely consistent with the view that spontaneous germination will proceed as the concentration in the soil falls below a limiting value. These experiments also show that, after the period of most rapid spontaneous germination is passed, the infectivity would appear to remain at a low but more or less steady value. It is presumably this residuum of resting spores which persists in the soil for long periods and is economically important in perpetuating the disease. It is perhaps this remaining fraction, of unknown size, which might respond to stimulation. As Brown (1946) has pointed out, in most known cases of dependent germination of spores, a proportion do germinate spontaneously, the number doing so varying widely with different organisms and different conditions, while the remainder requires biological stimulation. In one of the experiments with cropping treatments, cruciferous plants appeared to have effected such a stimulation and so reduced the number of viable spores in the soil. In the other experiment a similar result was obtained with *Lolium perenne*, so that the effect, if real, would not be entirely specific to crucifers. Reduced infectivity may apparently be achieved by other means, as indicated by the effect of merely adding nutrients to fallow soil. Moreover, in both these experiments soil conditions must have been changed considerably by the crop treatments, and hence effects on the spore population would always be confounded with the ultimate infection test. The results obtained, however, in view of their possible economic importance suggest that the influence of catch crops on the survival of *Plasmodiophora brassicae* might be worth investigating under more controlled conditions than hitherto, and over longer periods. While differences in numbers of root-hair infections were obtained by such treatments, in the field it is the number of clubbed plants which is of immediate interest. In Exp. 7 there were no differences in numbers of cabbage plants with clubroot, in spite of the considerable differences in root-hair infection. The experiments in which the root-hair infection count and the percentage of plants with clubroot were compared at different spore densities showed that high percentage clubroot could be associated with very low numbers of root-hair infections on young roots. It must, of course, be realized that each single root-hair infection gives rise to many zoospores, and this may represent an intermediate multiplication of the infecting agent. Furthermore, these root-hair infection counts were made only on a specified part of the seedling tap root and there is no information about the extent to which lateral roots were infected or how such infections might contribute to the development of taproot galls. An interesting effect of time of application and amount of nutrients was found in two of these experiments. It appeared that, in spite of similar numbers of root-hair infections, percentage clubroot was increased by early application and higher levels of nutrients. If this result can be confirmed, it would suggest that clubbing may be influenced by soil conditions subsequent to the first root-hair infections and that this influence might be important. Counts of clubbed plants

would, however, require to be made at a longer interval after the late application of nutrients than in these experiments since the effect might be merely one on the growth of clubs (cf. Walker & Hooker, 1945).

Webb's (1949) discovery of zoosporangia of *P. brassicae* in grasses and other non-crucifers, which was confirmed for such an important agricultural plant as *Lolium perenne*, raises the question of further means of multiplication and survival in the field. It is possible that the fungus can maintain itself for long periods in successive generations of zoosporangia in root hairs. From these roots it might in turn infect cruciferous weeds and there produce a fresh crop of resting spores. It should be pointed out, however, that it has not yet been demonstrated that the zoospores can re-infect the host and produce zoosporangia anew, though Ledingham's (1939) observations on *Polymyxa* would appear to suggest they may. Their role in the origin of the plasmodium in the galls is likewise obscure.

So far as the methods of assessing the activity of *Plasmodiophora brassicae* are concerned, mention has already been made of the scope and usefulness of percentage clubroot counts. In designing experiments so that this method can be used, it is apparent from Fig. 2 that infectivity must be adjusted to a suitably low level as judged by root-hair-infection counts. Such a standardization of infectivity, which would be valuable in all experiments, might be achieved by making use of the relationship found (Fig. 1) between spore concentration and numbers of root-hair infections. If the conditions employed were similar to those of the experiments described above, it would be possible, on the basis of a preliminary test with one spore concentration, to predict the density of infection at any other spore concentration with some accuracy. Even without such a test, a fair estimate of the order of infection might be made.

In the foregoing experiments, variation between replicates was considerable. When percentage clubroot counts were made, greater precision could have been achieved only by increasing the replication. In experiments using the root-hair infection-count technique modification of the sampling procedure by employing more replicate tumblers, but making counts on fewer seedlings from each, might have given more information per seedling examined. On the other hand, the extra tumblers would require increased labour for setting up and maintaining the experiment, more space, and so on. It was not thought worth while to do the costing necessary to evaluate the best sampling intensity from the available data, because the components of error variance obtained from Exp. 1 and shown in Table 10 are not estimated very accurately. In Table 11, however, rough estimates of the relative information per seedling examined are given for different sampling intensities because in practice it was the work of counting which effectively limited the accuracy possible. Thus, for example, by increasing the number of tumblers and making counts on 3 seedlings from each instead of 5 the accuracy for a given number of seedlings examined would be increased to $\frac{82}{70} \times 100 = 117\%$ of the present value. The number of tumblers, on the other hand, would be $\frac{5}{3} \times 100 = 167\%$ of the original

number so that, while it might in this way be possible to increase precision slightly, it does not seem likely to be economic to pursue this policy very far.

TABLE 10. *Analysis of variance and components or error variance for Exp.* 1

	D.F.	Mean sq.	Components of variance*
Treatments	34	72·939	
Between tumblers within treatments	105	0·195	$5\sigma_t^2+\sigma_s^2$
Between seedlings within tumblers	560	0·121	σ_s^2

* Where σ_t^2 = between replicate tumblers component of variance (single seedling basis) = 0·0148,
σ_s^2 = variance of a single seedling count = 0·121,

and variance of a mean based on n tumblers each containing r seedlings is

$$\sigma_{nr}^2=\frac{1}{n}\left(\sigma_t^2+\frac{\sigma_s^2}{r}\right).$$

TABLE 11. *Relative amount of information per seedling counted for different sampling intensities*

No. seedlings sampled for counting per tumbler	Relative information per seedling counted*
1	100
2	90
3	82
4	75
5	70

* Relative information $=\frac{\sigma_t^2+\sigma_s^2}{r\sigma_t^2+\sigma_s^2}\times 100$, where r = number of seedlings counted per tumbler; σ_t^2 = between replicate tumblers component of variance (from Table 10); σ_s^2 = within tumbler variance per seedling counts (from Table 10).

I am greatly indebted to Mr B. M. Church of the Statistical Department for advice and assistance on the statistical aspects of the work. Thanks are also due to Dr S. D. Garrett who introduced me to the subject.

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AN AUTOMATIC VOLUMETRIC SPORE TRAP

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(With Plate 11 and 2 Text-figures)

A suction trap has been made in which the spores entering a narrow orifice, directed into the wind, are impacted on a Vaseline-coated microscope slide moved across the orifice at 2 mm./hr. Estimates of spore content of the air can be made, with higher efficiency than by previous traps, at different times of day and thus be more closely correlated with variations in weather.

Wind-tunnel tests with spores of *Lycopodium clavatum* showed maximal and minimal efficiencies of 93·8 and 62·4% respectively, with a suction rate of 10·0 l./min., in the range of wind speeds from 1·5 to 9·3 m./sec.

Knowledge on the spore content of the air is important to research into the epidemiology of plant diseases, hay fever and asthma. The number of spores in the air has been estimated by various types of suction trap, and the exposure of sticky surfaces, chiefly planes or cylinders in various positions relative to the wind. A study of the action of sticky surfaces has recently been made by Gregory (1950), and the advantages of narrow vertical cylinders have been demonstrated. For research purposes in plant pathology, however, it is desirable to have a spore trap with a higher and less variable efficiency. Assessment of the number of spores in unit volume of air is likely to be inaccurate if the efficiency of a sticky surface (which depends partly on the wind speed) has to be calculated on the basis of the daily mean wind speed (see Johnson, 1950). It is also desirable to divide the day's catch into shorter time intervals, thus allowing closer correlation with weather changes.

Many of these difficulties could be reduced by the use of a suction-type trap which increases the efficiency of 'impaction' by artificially accelerating a narrow air stream towards a sticky surface. Movement of the sticky surface at a constant rate past the orifice through which this air-stream flows, should produce a trace showing changes of atmospheric spore content with time. Comparison of this trace with recording meteorological instruments would greatly increase the accuracy of its interpretation.

Suction traps employing the principle of impaction, some with movement of the sticky surface, have been described (Hawes, Small & Miller, 1942; May, 1945). Attempts have been made to show that the efficiency of filtration or impaction is high. Less attention has been paid to the efficiency of collection, i.e. whether the air-stream entering the trap bears a representative spore load. The conditions required for high efficiencies of both collection and filtration have been described by May (1945). The orifice should be directed into the air-stream, and the rate

of suction should produce a velocity in it equal to the external windspeed ('isokinetic sampling'). The Cascade Impactor described by May is a trap which can effectively fulfil these conditions. Under the controlled conditions of air-flow existing in a wind tunnel, it gives very high collection efficiencies. In the field its accuracy is reduced by the difficulty of matching suction rate to rapid changes of external wind speed during gusts. The best approximation is to maintain a constant suction rate and to make allowance for variations in collection efficiency caused by different wind velocities. Operation of a Cascade Impactor in the field and in the small wind tunnel at Rothamsted has shown that a complete removal of spores in the size range of pollens, rusts, powdery and downy mildews occurs with an orifice measuring 14 × 2 mm., providing that there is a suction rate of at least 6 l./min.

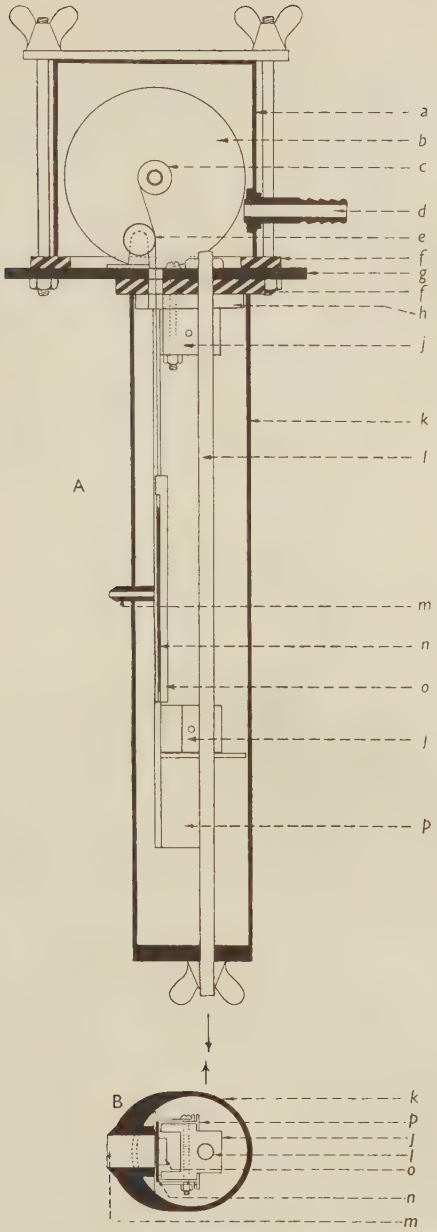
A trap has been constructed in which a sticky 76 × 25 mm. (3 × 1 in.) microscope slide is moved at 2 mm./hr. past an orifice 14 × 2 mm., to which suction at the rate of 10 l./min. is applied. A continuous trace of the day's spores is deposited on an area 48 × 14 mm. A wind-vane on the mounting directs the orifice into the wind. The orifice should be as short as possible and of the same bore throughout to avoid spore deposition on its walls. The mechanism for moving the slide should be as small as possible so as to avoid disturbance of the air at the orifice which projects 10 mm. from it. For convenience of comparison with meteorological instruments the orifice was placed at a height of 2 m.

Construction

The apparatus consists of three units: the impactor unit, the wind-vane mounting, and the motor housing.

The impactor unit (Pl. 11 and Text-fig. 1). This comprises the orifice and slide-moving mechanism. It is constructed of brass and extends above and below a 4 mm. (8 s.w.g.) brass mounting plate. This plate is used for securing the unit, and forming a backing against which rubber seals can be compressed to make the unit airtight. Below this mounting plate, the slide runway and orifice cylinder are mounted vertically, and above it is a box covering the clock operating the waxed thread which draws the slide and its carrier up the runway.

The slide runway is constructed of four lengths of $\lrcorner$ section brass, mounted in pairs, as shown in the cross-sectional drawing (Text-fig. 1 B). There is thus a channel 25 mm. (1 in.) wide and of adjustable thickness up which a microscope slide can travel. These pairs are separated and made rigid by being bolted to two solid brass spacing pieces. There must be no twisting in this assembly or the slide movement will not be uniform. Circular brass-positioning disks, which fit closely inside the orifice cylinder, are bolted to each of the spacing pieces. These disks ensure that the removable orifice cylinder is always correctly placed with reference to the slide. The upper spacing piece is bolted to the mounting plate through the upper positioning disk and through a 6 mm. ($\frac{1}{4}$ in.) sheet-rubber sealing disk of 6·6 cm. ($2\frac{1}{2}$ in). diameter. A 6 mm. ($\frac{1}{4}$ in.) brass tie rod, threaded at both ends, passes



Text-fig. 1. Diagrammatic sections of the impactor unit (one-third of actual size). A, vertical section; B, horizontal section at the level of the orifice. Parts of the air-tight shell and the slide, where cut by the plane of the section, are shown in solid black, except the rubber seals which are shaded. *a*, air-tight top cover; *b*, clock; *c*, thread drum; *d*, outlet tube; *e*, guide roller; *f*, rubber seal; *g*, mounting plate; *h*, positioning disk; *j*, spacing piece; *k*, removable orifice cylinder; *l*, tie rod; *m*, orifice; *n*, slide; *o*, slide carrier; *p*, slide runners of ⊥ section brass.

through the mounting plate, to which it is bolted, and through both spacing pieces. Its lower end is used to secure the orifice cylinder in position by a wing nut. The slide fits into the runway and is gripped at top and bottom by a carrier also engaged in the runway by means of tongues above and below the slide. Slide changing is facilitated by hinging the slide carrier at its centre.

The orifice cylinder is a brass tube 28·9 cm. (11 in.) long with an external diameter of 5·1 cm. (2 in.) sealed at its lower end with a circular brass plate drilled to allow the passage of the tie rod. The orifice is inserted at a point 12·7 cm. (5 in.) from the upper end of this tube. The internal dimensions of the orifice are 14 mm. horizontally and 2 mm. vertically. At its outer (upwind) end its sides are externally bevelled to a knife edge to reduce turbulence. For ease of construction and strength it is made with side lugs which are soldered externally to the tube. The inner end of the orifice has shoulders which rest on the forward edges of the slide runners and so increase the accuracy of its positioning. The clearance between the inner end of the orifice and the glass slide is slightly less than 1 mm.

The clock mechanism lies beneath an air-tight cover fixed on top of the mounting plate by four external rods and wing nuts. A thermograph clock is fitted with its centre in the vertical plane of the slide runway, and with its axis horizontal and at right angles to the direction of the wind. The slide carrier is moved by means of a thread, waxed to prevent hygroscopic changes in length. This thread is wound around a drum of 14·2 mm. ($\frac{9}{16}$ in.) diameter which is free to rotate about the clock spindle, but which is normally fixed to the spindle by a milled lock nut. Loosening of the lock nut allows adjustment of the slide position without interference with the clock. A small roller guides the thread through a hole in the mounting plate. The slide moves at 2 mm./hr. With an orifice also 2 mm. wide, the spores on any straight line parallel to the orifice long axis will represent the deposition during 1 hr. In practice the number of spores on narrow traverses is counted and the smallest interval of time at which spore content can be reliably estimated is 2 hr.

The trap can be maintained and the slide changed daily after the removal of the clock-mechanism cover and the orifice cylinder. When these are replaced and clamped into position the unit is air-tight, except for the orifice and an outlet tube on the rear or downwind side of the top cover. When suction is applied to the outlet tube a stream of air is drawn through the orifice, the spores are impacted on the slide and the air travels into the cover box by the same route as the thread which moves the slide. From the outlet tube a length of soft rubber semi-pressure tubing carries the air axially down the column of the mounting to the motor housing.

The wind-vane mounting. This unit has three functions; to raise the orifice to the operational height of 2 m.; to allow continuous rotation of the impactor so that the orifice faces the wind, and to provide a cover to prevent rain falling on the orifice.

The impactor unit is carried on a vertical pipe 6 ft. long with an external diameter of 6·6 cm. ($2\frac{1}{2}$ in.). At the top of the pipe a ball-race carries a smaller pipe surmounted by a flange plate to which the rain-guard and wind-vane assembly is

bolted. The rubber suction tubing passes down the common axis of the small and then the larger pipe. The rain-guard is a circular metal sheet 61 cm. (2 ft.) in diameter, the orifice cylinder is placed 10 cm. (4 in.) downwind of its centre.

The motor housing. This unit houses a vacuum pump and equipment for measuring the rate of suction, and acts as a stand from which the Impactor Unit can be serviced conveniently. It consists of a ventilated box mounted on legs sunk in the ground. The housing contains an Edwards Type IV Vacuum Pump, with a $\frac{1}{4}$ h.p. electric motor capable of continuous operation. The rate of suction is adjusted by a leak valve to give a pressure difference of 21·3 cm. (8·8 in.) in a water manometer placed in a branch of the suction tube. This pressure difference was found, by placing a flow-meter (Rotameter) in the suction line, to represent a flow rate of 10 l./min. through the orifice. Should the orifice become blocked the manometer liquid is sucked over into a small trap bottle which prevents its passage to the pump, and shows that the trap has not run normally.

Calibration of trapping efficiency

The sampling rate is not altered to match variations of external wind speed, and so the trap's collection efficiency at different wind speeds must be known. This was determined by comparing the Impactor Unit with other types of trap of known efficiency in a small wind tunnel under conditions of controlled turbulence. Tests were made at each of four external wind speeds, using two rates of suction through the orifice. The test particles used were spores of *Lycopodium clavatum*, because they are easy to handle and much is known about their behaviour as air-borne particles. Their size and terminal velocity make them suitable for a standard of comparison with other spores in the size range mentioned above. Smaller and lighter spores would be expected to show a higher collection efficiency which would be less subject to variation with external wind speeds. Impaction efficiency would, however, be lower, unless the size of orifice was reduced or the suction rate increased.

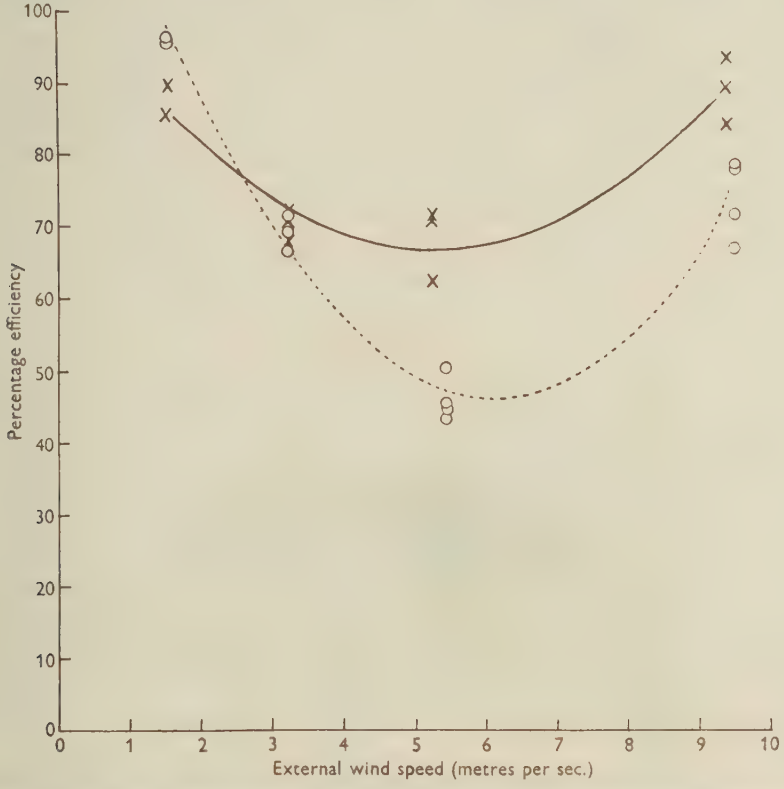
The distribution of spores caught on the slide, which was stationary throughout these tests, was not uniform over the area below the orifice. This difficulty is mentioned by May (1945) in connexion with the operation of the Cascade Impactor in still air, when the sampling cannot be isokinetic. He stated that the distribution can be made more uniform by placing a bell mouth in front of the orifice. Such an addition is not possible with a trap which is also to operate in high winds, as it would cause much disturbance at the orifice. In the open air the small differences which occur along the short (2 mm.) axis of the orifice are obviated by the continuous movement of the slide. More important irregularities occur along the long axis of the orifice, but these are not apparent when traverses are made along this axis to determine the atmospheric spore content at different times. Furthermore, in the field the rapid changes in wind direction and velocity at the orifice caused by gusts tends to equalize the deposition.

In the wind-tunnel tests there were two areas of dense deposit along the long axis of the orifice. Their position varied with the suction rate and the external wind speed. The distribution of the spores along the long axis of the orifice was determined for each set of conditions. From these data, correction factors were calculated, which enable an estimate of the total number of spores caught on the slide to be obtained from counts of predetermined areas along the long axis. Efficiency was calculated as the percentage of the spores present in a cubic metre of air which were caught by the trap. The mean number of spores present per $m.^3$ was estimated by simultaneously exposing vertical cylinders of 0·53 cm. diameter (Gregory, 1951), at the three highest wind speeds. At the lowest wind speed, 1·5 m./sec., the cylinder was replaced by the Cascade Impactor, because vertical cylinders are inefficient at this low wind speed and a deposit on the cylinder dense enough to give reliable counts meant that many spores had to be liberated and the trace in the suction trap was then too dense to count.

Text-fig. 2 shows the results of these tests, using four external wind speeds 1·5, 3·2, 5·2 and 9·3 m./sec., and two rates of suction, 10·0 and 17·5 l./min. The curves which best fit the results of the tests have been calculated and apply only to wind speeds in the range tested. Their shape may have to be modified when tests are made at additional wind speeds. The rate of 10·0 l./min. was chosen for operation in the field, because efficiency varied less at different wind speeds than at 17·5 l./min. There was also less danger of obscuring the spore deposit with fine dust particles which are less efficiently impacted with a lower velocity in the orifice.

The impaction of *Lycopodium* spores, pollens, rust and mildew spores should be complete at either of these suction rates. This is confirmed by the fact that there was no scattering of *Lycopodium* spores on the sticky surface outside the orifice area. This is very important if the time basis of deposition in the trace is to be reliable. Thus an efficiency approaching 100% would be expected in still air and also when the conditions of sampling were isokinetic. Suction rates of 17·5 and 10·0 l./min. produce velocities in the orifice of 10·4 and 6·0 m./sec. At the higher suction rate the theoretically perfect isokinetic sampling conditions were never reached in the range of wind speeds tested. At the lower suction rate of 10·0 l./min. however, the high efficiency of collection expected with an external wind speed of 6·0 m./sec. was not found. May states that when operating the Cascade Impactor at a suction rate of 17·5 l./min., thus producing a velocity of 2·2 m./sec. in the first orifice, 'flow patterns into the orifice are most satisfactory when the main air stream is moving at about 3·5 m./sec.'. This he attributes to the fact that the streamlines, approaching the vertical cylinder which bears the orifice, are slowed down as they come close to the cylinder. He considers that the position of the impactor orifice is such that it projects forwards into a region where the velocity has fallen to about two-thirds of its original value, where with the suction rate in use the sampling velocity would be approximately isokinetic. The Automatic Volumetric Trap has a cylinder approximately twice the diameter of the Cascade Impactor, the forward

projection of its orifice from the foremost part of the cylinder is also twice that of the Cascade Impactor. If we apply the same factor to the orifice velocities used in the Automatic Volumetric Trap, we should expect the highest efficiencies to be reached with wind speeds of 9 and 16 m./sec. with suction rates of 10·0 and 17·5 l./min.



Text-fig. 2. Efficiency of trapping of *Lycopodium clavatum* spores with external wind speeds in the range 1·5–9·3 m./sec. Observed values from wind tunnel tests: ×, suction rate 10·0 l./min.; ○, suction rate 17·5 l./min. Curves of best fit to the observed values, solid line for suction rate of 10·0 l./min., dotted line for suction rate of 17·5 l./min.

respectively. The results of the wind tunnel tests in Text-fig. 2 substantially agree with this expectation. In the open air the Automatic Volumetric Trap is operated near to an anemometer, also at a height of 2 m., which continuously records wind run against time. It is thus possible to apply corrections for the efficiency of the trap on the basis of the mean wind speed over any desired time interval.

Adhesives and mounting

The requirements for substances suitable for the sticky surface and for making of permanent preparations for microscopical examination, are exacting. The sticky surface must permit a high efficiency of impaction; this requires a soft, almost wet surface, which is not hygroscopic or subject to drying during the period of exposure. It must have good optical properties, allow the swelling of shrunken spores, so they can be identified and not be incompatible with other substances used in the making of permanent preparations. Of the substances so far used none was wholly satisfactory, and the best was Vaseline. Glycerine jelly was rejected because it is very hygroscopic and not easy to mount without some movement of the spores. It was thought likely that the gelatine was the cause of its hygroscopicity, and a mixture of two-thirds 2% agar and one-third glycerol was tested; this was equally difficult to mount, and fine droplets caught in misty conditions caused considerable spore movement. Silicone grease gives a high impaction efficiency and has good optical properties, but the spores are rapidly wetted by the grease and any shrinkage of the spores is irreversible.

Many of the usual methods of mounting detach the spores from the sticky surface, others emulsify the Vaseline layer or fail to allow the swelling of the spores. The method at present in use gives good results but requires considerable care. A thin film of Vaseline about 16 mm. wide is applied to the area of the slide which is to pass under the orifice. After exposure the slide is gently warmed on a hot-plate for about 5 min., so as to soften but not to melt the Vaseline film. This allows the particles to become more firmly attached, so preventing any movement later. The exposed area is then lowered on to a 51 × 22 mm. ($2 \times \frac{7}{8}$ in.) cover-glass bearing just sufficient lactophenol to make a film between the cover-glass and the slide. The edges of the cover-glass are then sealed to the slide with Wallers Cement no. 1 (Dade & Waller, 1949) and care must be taken not to overheat the cover-glass and so melt the Vaseline film beneath. The spores take a day or two to regain their original shape.

The trap has been continuously operated for several months; the results have not yet been fully analysed, but they provide clear evidence of large variations in atmospheric spore load, both diurnally and as a result of rain.

I wish to thank Mr J. H. A. Dunwoody for the calculation of the curves shown in Text-fig. 2.

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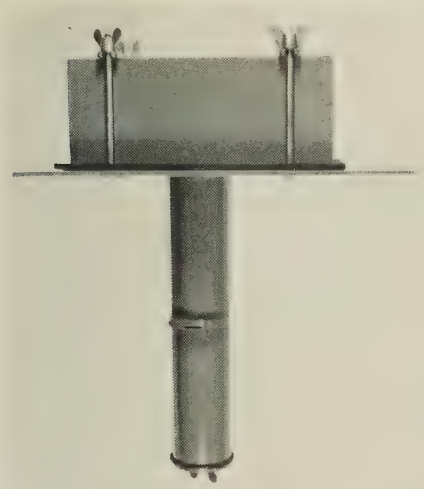

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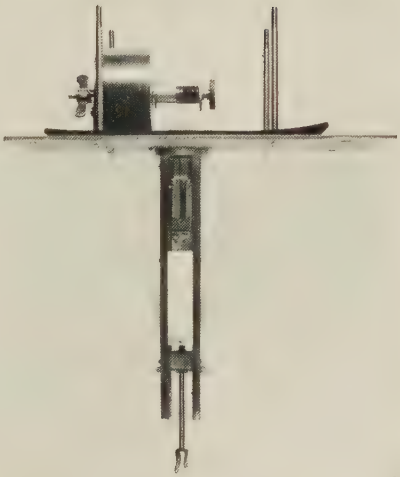

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EXPLANATION OF PLATE 11

Fig. 1. External view of impactor unit, from upwind, with top cover and orifice cylinder in position.

Fig. 2. View of the impactor unit from upwind side, after the removal of the top cover and the orifice cylinder. A white card has been substituted for the microscope slide.

Fig. 3. General view of the trap in operation in the field.

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SOME EFFECTS OF NITROGEN NUTRITION ON THE MORPHOLOGY AND ANATOMY OF MARROW-STEM KALE

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(With 1 Text-figure)

Marrow-stem kale plants grown on plots receiving frequent additions of sulphate of ammonia showed a 40% increase in length of internode and a 25% increase in number of nodes per plant, and the leaf size was increased by between 50 and 70% over plants in plots receiving no N fertilizer. Leaves of kale continue to increase in area until they turn yellow, and the high N leaves showed a greater rate of increase in area at every stage in the life of the leaf.

Various features of leaf structure, such as stomatal index, and thickness of palisade and mesophyll, were unaffected by N treatment. The size of the epidermal cells of the leaves was very variable, and although the high N leaves showed a 12% increase in area per epidermal cell over the low N leaves, this difference is not statistically significant. The increased area of the high N leaves can therefore be attributed mainly to increased cell division during the life of the leaf. Only a very slight increase in rate of cell division is necessary to produce the observed effect.

The greater leaf area of the high N plants can be attributed mainly to increased size of individual leaves, but there was also a significantly greater number of living functional leaves per plant on the high N plants; at 23 weeks from sowing the high N plants had an average of 13·4 living leaves, while the low N plants had only 11·7 living leaves per plant.

There was an appreciable degree of N succulence in the high N kale leaves, which showed a 2% greater moisture content than the low N leaves.

A seasonal drift in epidermal cell size, palisade thickness, and total leaf thickness, is shown to be fully significant, statistically. Marked variations in stomatal frequency are barely significant at the 5% level.

Introduction

It is obvious that extreme N starvation will seriously restrict the growth of plant organs, through a reduction of protein synthesis and the consequent restriction of meristematic activity. This effect largely accounts for the stunting of plants exposed to extreme N starvation—for example in N-free water culture or sand culture. Conversely, a high N supply might be expected to favour protein synthesis, and thus to increase meristematic activity, leading to abnormal luxuriance. The literature does not provide conclusive evidence that the increased growth which occurs in all plant organs at higher N levels is solely the result of increased meristematic activity. It appears that there is often an appreciable increase in cell size in leaves, which could account for some, but not all, of the increased leaf size. Morton

& Watson (1948), and also Mothes (1932), both showed that the size of the leaf cells was increased by raising the N level, but in both investigations the leaf size was increased proportionately much more than the cell size, so that higher N levels also increased the *number* of cells per leaf, which would indicate increased meristematic activity, either in the young leaf primordium, or at the stem apex (increasing the number of cells initially included in each leaf primordium).

The effect of N level on the plastochron interval suggests increased activity of the apical meristem at higher N levels. Crowther (1934) found that applications of N to the cotton plant produced a 16% increase in number of internodes at 3 months after sowing, and a 36% increase at maturity; there was no effect on the length per internode. Batger & Degman (1940) grew apple trees in sand cultures at five different concentrations of N, varying from 0 to 168 p.p.m. The length, and number, of internodes composing the shoot are not given, but estimates from the scale photographs accompanying the paper show that there is no appreciable difference in the mean length per internode over the whole range of N levels, although the length of the leading shoots was trebled over this range of N levels. Applications of N to the sugar-cane increased the rate of formation of leaf initials, but had no effect on the length of each internode (Das, 1936). On the other hand, Garner, Bacon, Bowling & Brown (1934) found that an application of 20 lb./acre of nitrate of soda to tobacco plants decreased the number of internodes differentiated, while the mean length of internode was increased by about 28%. There was little net effect of N level on the height of the stem.

The possible role of N succulence must not be overlooked, for the increased water content of plant organs under high N conditions will obviously contribute to the increased bulk of the plants grown at these high N levels (see especially Pearsall & Ewing, 1929). The magnitude of the effect is usually about 2% in the water content of the tissues, and on this scale it will not be a major factor, compared with cell size and cell number.

The effect of N nutrition on growth may be modified considerably by changes in the amount of carbohydrate available. The decreased growth which sometimes results from applications of N under conditions of low light intensity is fully discussed by Nightingale (1948).

Experimental detail

Samples of kale leaves were obtained from trial plots at the National Institute for Research in Dairying near Reading, in which the layout was designed to cover spacing as well as N level. Three different spacings and two N levels were combined, giving six treatments replicated in four blocks. Only the plots in which the plants were thinned to 9 in. between the plants in the row were used; the rows were 25 in. apart.

The seed was sown on 13 May 1946, and the ground had not had any previous

applications of nitrogenous fertilizer in 1946. On 11 April salt was applied at the rate of 4 cwt./acre. The low N plots received no application of N fertilizer during the whole trials period, while the high N plots received three applications of sulphate of ammonia, each at the rate of 2 cwt./acre, on 30 May, 18 June and 8 July. This difference in N treatment had a pronounced effect on the N content of the crop, as the crude protein content of the first harvest (at the end of November) shows. I am indebted to the National Institute for Research in Dairying for permission to publish the following results of their analyses of the 9 in. spaced crop, showing the following percentages of crude protein in the dry matter: leaf—high N 20·7%, low N 15·3%; stem—high N 11·7%, low N 6·2%.

Leaf samples were taken, and growth measurements made, at intervals between 10 July (57 days after sowing) and 21 October 1946. The leaves were cut from the plant at the base of the petiole and the cut end dipped under water immediately. The cut leaves were then packed into large battery jars with a little water at the bottom, and covered with a damp cloth, for transport back to the laboratory. Usually less than 2 hr. elapsed between the severing of the leaves and the commencement of operations in the laboratory. Leaves were numbered in serial order as they appeared on the plant, not counting the cotyledons. Epidermal strips were removed from the upper epidermis in two places chosen at random on different sides of the midrib, and different ends of the leaf. The areas chosen for examination and measurement were always clear of veins, and well away from the main veins. Tests showed that the area of the epidermal cells was not appreciably different in water or in dilute glycerin, so that there can have been little turgor stretching of the cell walls as a result of the turgor of the epidermal protoplasts; no information was obtained on the amount of stretching imposed on the epidermis in the intact leaf as a result of the turgor of the *mesophyll* cells. The measurements given in the tables are from epidermal strips mounted in dilute glycerin, and are therefore appropriate to non-turgid cells. An area of the camera lucida drawing delimited by cell walls was measured with a planimeter, and the number of epidermal cells and stomata counted. Transverse sections were cut from rectangles of tissue taken from the centre of islands between the larger veins. The thickness of the palisade and mesophyll was measured at six or seven places along two or three sections from each rectangle, and the values averaged.

The stem growth of kale

The results summarized in Table 1 show that the applications of N almost doubled the elongation growth of the stem by the time the plants were 15 weeks old. The total number of leaves produced by each plant was increased by about 23% as a result of these applications of N, and the remainder of the difference in stem elongation is attributable to the greater mean length per internode in the high N plants. All these differences are highly significant (1% level).

TABLE 1. *Stem growth and leaf production in kale plants, after 15 weeks' growth*

(Figures represent the means of ten plants.)

	Length of stem (in.)	No. of elongated internodes	Mean length of internode (in.)	Total no. of leaves per plant
Low N	11·4	12·5	0·92	16·5
High N	20·5	16·6	1·24	20·3
Difference	9·1	4·1	0·32	3·8
S.E. of difference	1·44	0·46	0·11	0·61
Level of significance (%)	0·1	0·1	1·0	0·1

LEAF GROWTH OF KALE

Kale leaves continue to increase in area until they turn yellow, as the results summarized in Table 2 show. The high N leaves increased in length even more than the low N leaves over the same 10-day period, although the high N leaves were more senile than the low N leaves at this stage. This result is fully significant at the 1% level.

TABLE 2. *Growth of the 7th leaf between 18 July and 30 July*

(The +N leaves began to die on 30 July; the −N leaves on 14 August. Measurements taken on nine leaves from nine different plants for each treatment. The same leaves were used on both dates.)

Length (cm.)			Breadth (cm.)		
18 July	30 July	Growth	18 July	30 July	Growth
		With N			
26	36	10	22	27	5
26	29	3	25	28	3
22	25	3	17	21	4
32	40	8	25	30	5
28	30	2	23	26	3
25	30	5	23	30	7
30	34	4	20	23	3
29	32	3	26	28	2
25	28	3	27	30	3
27·0	31·6	4·6	23·1	27·0	3·9
Mean increase in leaf area between 18 July and 30 July: 38% ± 6%					
		Without N			
30	32	2	21	24	3
24	24	0	16	16	0
22	23	1	17	19	2
20	21	1	14	15	1
23	25	2	17	20	3
20	22	2	16	19	3
23	22	−1	23	23	0
18	22	4	15	16	1
25	26	1	20	20	0
22·8	24·1	1·3	17·7	19·1	1·4
Mean increase in leaf area between 18 July and 30 July: 16% ± 4%					

The fact that leaves of kale continue to grow throughout their whole lifetime complicates the comparison of leaf areas between the two N treatments. But since

the low N leaves grow much less rapidly in the later stages, differences in size resulting from the N treatment will be minimized rather than exaggerated by this complication, provided that the comparisons are made between leaves of approximately the same ages.

Table 3. *The effect of level of* N *supply on the length and breadth of mature leaves of kale*

(All figures represent the mean of at least ten (sometimes twenty) leaves on separate plants.)

		High N				Low N				
Date of measurement	Serial order of leaf on stem	Age of leaf (days)	Expected date of death (from Fig. 1)	Length (cm.)	Breadth (cm.)	Age of leaf (days)	Expected date of death (from Fig. 1)	Length (cm.)	Breadth (cm.)	Ratio of leaf areas: High N / Low N
20 June	1	20	25 June	7·3	4·7	20	25 June	6·1	4·5	1·26
20 ,,	2	16	30 ,,	9·0	6·8	16	30 ,,	7·9	5·9	1·31
18 July	5	24	25 July	25·0	20·0	24	25 July	22·0	18·0	1·26
18 ,,	6	19	30 ,,	25·5	23·2	19	30 ,,	23·3	17·7	1·43
18 ,,	7	16	6 Aug.	27·3	23·2	16	6 Aug.	23·1	17·8	1·54
30 ,,	7	28	6 ,,	31·5	27·0	28	6 ,,	24·0	19·0	1·82
30 ,,	8	24	14 ,,	31·0	26·5	23	15 ,,	24·8	19·0	1·75
30 ,,	9	20	21 ,,	32·0	27·1	14	30 ,,	24·5	19·2	1·82
30 ,,	10	16	26 ,,	32·4	28·1	10	7 Sept.	22·3	17·2	2·30
28 Aug.	11	—	—	—	—	32	25 ,,	26·2	20·2	1·50
28 ,,	13	33	12 Sept.	30·3	26·6	—	—	—	—	
21 Oct.	17	—	—	—	—	51	15 Nov.	24·4	17·4	1·60
21 ,,	20	57	6 Nov.	29·6	22·7	—	—	—	—	

In making comparisons between the leaf areas of the high N leaves and the low N leaves (data in Table 3), it is essential to distinguish three separate phases of the growth cycle:

(A) Leaves 1–6 inclusive, in which leaves with corresponding serial numbers develop approximately simultaneously at both N levels. The high N leaves in this phase have leaf sizes 1·3 times as great as the corresponding low N leaves.

(B) Leaves 7–10 inclusive, in which the low N leaf is considerably younger than the high N leaf with which it is compared, at the time of comparison. The low N leaf might therefore be less fully expanded than the corresponding high N leaf at the time of measurement. This is particularly likely in the case of leaf 10, where the low N leaf was only 10 days old at the time of measurement, while the high N leaf was 16 days old, so that the exceptionally high ratio: high N area/low N area for this leaf must be regarded with suspicion.

(C) Leaves 11, 13, 17 and 20, in which the comparison between the two N levels is based on leaves of the same stage of maturity but of different serial orders. The oldest leaf on the plants of each treatment was chosen for comparison, but not all the leaves of the two serial orders were on the point of dying at the sampling date. These comparisons give leaf area ratios which are intermediate between those of phases A and B.

It seems fair to conclude that there is a slight upward trend in the leaf area ratio between the two N levels, as the plants get older. The increased N supply has thus had a very marked effect upon the individual leaf size of the kale plants, producing an increase of at least 50% over the low N leaf size. This effect provides the major contribution towards the increased leaf area per plant resulting from the increased N supply, but the results summarized in Table 4 indicate that the number of living, mature leaves on each high N plant at any one time during the growing season is slightly greater than the corresponding number of leaves on the low N plants. Taken individually, the differences on 28 August, and on 21 October are not statistically significant, but the overall means for both dates combined shows a difference which is fully significant at the 1% level. In approximate terms we may say that the high N plants show a 10% increase in the number of functional leaves per plant over the low N plants, and that these leaves are individually 1·5 times as large as the low N leaves.

TABLE 4. *The number of living and functional leaves per plant*

(Figures are the means of ten plants; partially expanded leaves are included.)

	28 Aug.	21 Oct.	Aug. and Oct. pooled
High N plants	10·2	13·4	11·8
Low N plants	8·7	11·7	10·2
Difference between high and low N	1·5	1·7	1·6
S.E. of difference	1·6	1·1	0·5
Significance	Not significant	Not significant	Fully significant (1%)

TABLE 5. *The effect of* N *level on the relation between leaf area, fresh weight, and dry weight*

Sampling date	N level	Leaf serial no.	Leaf area product	Fresh wt. per leaf (g.)	Dry wt. per leaf (g.)	Water content (% fresh wt.)
28 Aug.	+N	13	8·05	30·6	4·32	85·9
	−N	11	5·3	17·7	2·85	83·9
	+N/−N	13/11	1·5	1·73	1·52	1·03
21 Oct.	+N	20	6·71	18·2	2·85	84·4
	−N	17	4·25	12·5	2·16	82·8
	+N/−N	20/17	1·58	1·43	1·32	1·02
21 Oct.	+N	23	—	13·4	2·18	83·7
	−N	20	—	10·6	1·87	82·4
	+N/−N	23/20	—	1·27	1·16	1·03
21 Oct.	+N	26	—	12·6	1·98	84·3
	−N	23	—	6·1	0·96	84·4
	+N/−N	26/23	—	2·1	2·6	1·0

Table 5 gives data on the effect of N supply on the fresh weight and dry weight of the leaves. The differences between the two N levels parallel almost exactly the differences in leaf area, as would be expected. The high N leaves show a consistently higher water content than the low N leaves, and the magnitude of this

effect is of the same order as the 'nitrogen succulence' found in other plants—Crist (1926), Pearsall & Ewing (1929), Carroll (1943), and Tam & Clark (1943). It may be noted that the dry matter per unit leaf area is the same for both N levels on 28 August, and is slightly greater in the low N plants than in the high N plants on 21 October. This may be taken as an indication that the moderate N deficiency in the low N plants has not reduced the net assimilation rate of these plants.

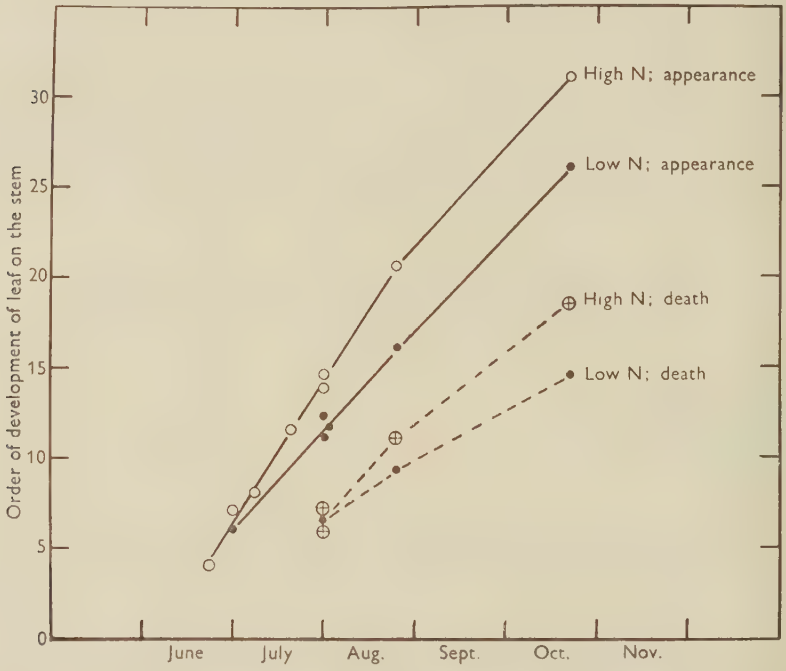

Fig. 1. The dates of appearance and death of kale leaves (death = onset of yellowing).

There appears to be very little difference in the longevity of the leaves at the two N levels, and this can be seen in Fig. 1. Richards & Templeman (1936) also found only a slight reduction in the length of life of leaves on N-deficient barley plants.

Seasonal changes in leaf anatomy

A detailed analysis of variance of the data in Table 6 shows that there is a significant (1% level) effect of season on epidermal cell size, palisade thickness, and total leaf thickness. The seasonal variations in the other characteristics measured are not significant; neither is the effect of N level, except in the case of leaf size. There is no interaction between N level and season. The marked variations in stomatal frequency are barely significant at the 5% level, but, as would be expected, they closely parallel the variations in epidermal cell size.

Although there is a very considerable change in leaf size (leaf area product, Table 6) as the season advances, this drift is only significant at the 5% level because of the great variability in leaf size. The increase in leaf size during July is accompanied by a decrease in size of the epidermal cells, so that the total number of cells per leaf must increase very considerably during this part of the season. The inverse relation between leaf size and cell size also holds during the latter part of the season, when leaf size decreases and cell size increases, but it must be remembered that these autumnal changes are not statistically significant.

TABLE 6. *The effect of* N *level, and of season, on the leaf anatomy of kale*

(The figures represent the mean of ten leaves, and the S.E.'s of the means. Vertical bars connect means which are significantly different (5% level), while a double vertical bar indicates a highly significant (1% level) difference.)

Date of sampling		'Leaf area product' Length × Breadth	Leaf thickness (including the epidermis) Palisade (μ)	Mesophyll (μ)	Total (μ)	No. of epidermal cells/mm.², leaf area	Stomatal index	Stomatal frequency
10 July	+N	420±60	250 —	160±20	410±20	301±20·6	21·8±1·1	83± 6
	−N	315±40	300±40	190±12	490±40	386±62	22·2±0·4	110±20
18 July	+N	475±36	310±13	190± 5	500±14	387±53	20·6±1·1	97±11
	−N	412±32	300±15	180±10	480±38	393±59	21·9±1·0	111±15
30 July	+N	|| 787±57	220±21	170±11	390±27	555±45	24·4±1·0	132± 8
	−N	|| 525±26	190±14	160± 6	350±20	621±35	24 ±1·4	148± 9
28 Aug.	+N	|| 802±61	170±13	160± 4	330±16	| 747±54	21·7±1·0	| 161±12
	−N	|| 539±50	170± 7	160± 5	330±12	| 931±51	22·8±0·7	| 213±15
21 Oct.	+N	|| 682±64	| 140± 7	| 150± 5	| 290±12	698±32	19 ±1·0	132± 8
	−N	|| 427±36	| 160± 4	| 170± 4	| 330± 8	678±37	19·5±0·9	133±10
Overall means	+N	| 633±57	220±14	170± 7	390±18	538±45	21·5±1·1	121±10
	−N	| 470±39	220±13	170± 7	390±19	602±47	22·1±1·0	143±13
Mean of both N levels taken together for each date:								
10 July		369±36	275±23	175± 9	450±25	343±33	22·0±1·1	96±10
18 ,,		|| 444±24	|| 305±32	| 185± 6	|| 495±14	|| 390±40	| 21·3±0·7	|| 104± 9
30 ,,		|| 656±30	|| 205±12	| 165± 6	|| 370±16	|| 588±34	| 24·2±0·9	|| 140± 6
28 Aug.		| 670±40	| 170± 7	160± 3	330±10	|| 839±37	|| 22·3±0·6	|| 187±10
21 Oct.		| 555±37	| 150± 4	160± 3	310± 7	|| 688±24	|| 19·3±0·7	|| 133± 6

The increase in leaf size during July (Table 6) refers to the 4th, 5th, 7th, 8th and 9th leaves after the cotyledons at approximately comparable stages of maturity. It is quite possible that this progressive increase in leaf size from the 5th to the 9th leaf is really ontogenetic in origin rather than seasonal. In any case, the drift in cell size shows that the increase in leaf size during this period is entirely brought about by increased meristematic activity in the leaf primordium or at the stem apex. In this respect the phenomenon may be somewhat different from the increased leaf size brought about by increasing the N level, to be described in the following section.

The effect of N level on leaf anatomy

Table 6 shows that the N level has practically no effect on the thickness of leaves and relative proportions of palisade and mesophyll, or the stomatal index or stomatal frequency. This agrees with the work of Mothes mentioned earlier.

Although the epidermal cell size of the high N leaves is consistently greater than for the low N leaves on each occasion except 21 October, yet statistical analysis shows that this difference is not significant. This should not be taken as proving that there is no difference in cell size between the two N levels, for the great variability in cell size from plant to plant has much to do with the lack of significance. The overall mean values show that, in the samples measured, the high N cells were, on the whole, 1·1 times as large as the low N cells. A similar effect of N level was demonstrated by Mothes (1932) and by Morton & Watson (1948). It remains possible, therefore, that the larger leaf size of the high N leaves is at least partly the result of increased cell size, although increased cell division must play an important part as well. In this respect the effect of N level on leaf size may partly spring from a different source from the effect of season already described.

Discussion

The published results for cotton, sugar-cane, and apple trees (Crowther, 1934; Das, 1936; Batger & Degman, 1940) all agree that higher N levels increase the rate of formation of leaf primordia, and so result in a greater number of internodes per plant. On the other hand, the tobacco seems to react to increased N level with decreased number of longer internodes. The results obtained with kale are intermediate, in that they show an increase in both number of internodes and in length at the higher N level. Thus the increased vigour of shoot growth which is so generally associated with higher N levels is partly a reflexion of the shorter plastochron period, but may also, in some plants, be associated with increased internode length. The growth habit of the kale plant rules out any question of increased number of branches. In the absence of any information on cell size in the kale internodes it is impossible to draw any conclusions about the part played by meristematic activity in developing this difference in length. The simplest explanation of the shorter plastochron period in the high N plants is that the N supply has increased the rate of cell division at the apex.

The ratio of the sum of the areas of all the high N leaves in Table 3 to the sum of the areas of all the low N leaves in that table is 1·7, while the ratio of the total length of stem in the high N and low N plants is 1·75, so that the very marked effect of moderate differences in N level on the growth of kale is almost the same in both stem and leaf. It is valid to base this comparison on leaf area and stem length provided that N level has no effect on the number of cells in a cross-section of the stem immediately behind the apex (unfortunately there is no information on

this point). The diameter of the mature stem does not enter into this comparison unless we wish to assess the effect of N level on cambial activity alone.

The work of Watson & Russell (1943) on mangolds, and of Woodman & Paver (1944) on turnips, shows that changes in the N level may affect different parts of the plant to differing degrees. The ratio of fresh weight of roots to that of tops was changed from 3·6 to 4·5 by additions of N to mangolds, while an increase in N level from 4 to 66 p.p.m. in turnips gave a tenfold increase in fresh weight of tops and a thirteenfold increase in the fresh weight of roots. Unless this differential effect is the result of larger cell size in the high N plants, it appears that the meristematic activity in the leaf primordia is less affected by N level than the cambial activity of the roots. No measurements of stem thickness were made on the kale plants, so that the effect of N level on the cambial activity of the stem cannot be assessed.

The degree of N starvation obtained in the kale plants during this trial was only very moderate, and the differences in leaf area at the two N levels were nothing like as large as those reported in other plants for wider differences in N level. The effect of season on both leaf size and number of cells per leaf in the kale trials is greater than the effect of the N levels employed. The seasonal effect may be comparatively large, even over short periods of time, as for instance between 18 and 30 July, or between 28 August and 21 October. The number of cells per leaf in the leaf maturing by 30 July was more than double the number of cells per leaf in leaves maturing on 18 July. But it is important to realize that this difference can be brought about by only one extra cell division, or generation of cells, occurring between the original cells of the leaf primordium and the mature cells of the expanded leaf. This seasonal effect far surpasses anything obtained by varying the N level at any time during the experiments, and it is important to bear in mind the comparatively insignificant change in rate of cell division which would be sufficient to account for the quite appreciable differences in number of cells per leaf, and total leaf area, which were recorded from the two N levels. At the same time, however, the very considerable effect which this slight difference in rate of cell division does produce is an indication of the great difference in consumption of materials, protoplasmic and otherwise, which accompanies such a slight difference in rate of division. No doubt wider differences in N level would have produced even bigger differences in leaf area and cell number.

It is a pleasure to express gratitude to the Director of the National Institute for Research in Dairying for access to the trial plots, and for permission to publish relevant analytical data obtained by the research institute staff; and to Mr A. S. Foot for helpful and painstaking co-operation at many stages. The investigation was carried out in the Botany Department at Southampton under Prof. S. Mangham, to whom I owe gratitude for much more than his encouragement in this research.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

General Meeting of the Association held at King's College, Newcastle-upon-Tyne on Tuesday and Wednesday, 8 and 9 January 1952; the President, Mr R.W. Marsh, in the Chair.

After the formal business, the following papers were read and discussed:

1. Phenological aspects of egg hatching mechanisms in Homoptera. By Dr. G. Heslop-Harrison.
2. Notes on inspection methods for interpreting field data. By Dr J. Macleod.
3. The potato-root eelworm cyst as a hatching unit. By Dr C. Ellenby.
4. Recent work in Ayrshire on the control of potato-root eelworm. By Dr John Grainger.
5. Field observations on timothy flies (*Amaurosoma* spp.). By Mr K. J. Coghill.
6. Nickel toxicity in plants. By J. G. Hunter and O. Vergnano.
7. The effectiveness of native strains of the field bean nodule organism. By Miss J. Baird.

RECENT WORK IN AYRSHIRE ON THE CONTROL OF POTATO-ROOT EELWORM

By John Grainger, *West of Scotland Agricultural College, Auchincruive, Ayr*

Early potatoes are grown for ware in a narrow coastal belt of Ayrshire and Wigtownshire. The variety is invariably Epicure, and most of the fields are infected with potato-root eelworm, *Heterodera rostochiensis*. Crops are grown year after year, for, in spite of an annual loss of about 40% in yield of potato tubers, the crop is of high value and the practice is still remunerative. Infections of the soil with eelworm cysts are not heavy and foliage symptoms are slight, for the crop is planted early, it makes quick growth, and is lifted early. Growth begins at a time when soil temperatures are low, so that presumably the hatching of larvae in the presence of the crop is delayed. Cysts are, moreover, mostly white and probably immature when lifted, and may therefore not make a large annual contribution to the cyst population of the soil. When Epicure is grown later in the year, it suffers a greater diminution of yield (about 80%), foliage symptoms appear early in growth, and become severe, and cysts are brown (mature) at the time of lifting.

The natural rise and fall of eelworm populations in the soil was measured in pot experiments, and checked by field estimations. When potatoes were grown, the greatest increase (52,000 cysts per plant) occurred when the initial infection was 0·8 cyst with contents per gram of soil. Rates of increase diminished with initial infections higher and lower than this value, the relation being portrayed by an asymmetrical modal curve having its maximum at 0·8 cyst per gram of initial infection. When potatoes were not grown, the cyst population wasted naturally at the rate of 30% per year, in pot experiments, and at something below

20% in the field. This explains the long persistence of the disease in the absence of a susceptible crop. It also shows that control by rotation could only be accomplished by lengthening the period between potatoes to a degree impossible in practice.

Growth-cycle studies confirmed the very severe effects on yield, and the depressing effects of the eelworm on growth could be seen from the earliest stages. Percentages of total carbohydrate and nitrogen were similar in healthy and diseased plants, but percentages of ash in eelworm-infected plants were only about half those in healthy growth. Severity of eelworm infestation of the soil was independent of pH, and of the amounts of phosphate, potash, loss on ignition, total salt content and lime requirement to pH 6·25.

Experiments on possible control have been made with a variety of substances, but only two have been selected for practical development. Mercury compounds achieve control at low cost for materials, but they must await the design and production of a machine capable of mixing them thoroughly enough with the soil. DD mixture (of dichloropropane and dichloropropylene) is more expensive, but can be applied with relative ease. It is injected into the soil in traces up to 6 in. deep and about 12 in. apart, where it evaporates slowly, and the vapour penetrates the soil mass.

The annual potato crops of Ayrshire are followed by catch crops of Italian ryegrass or other green forage. Soil temperatures are, moreover, low in winter, and, with the occurrence of a large number of rain days, make it impracticable to give treatment in the dormant season. It was therefore found necessary to inject in June or July, immediately after a crop was lifted, to benefit the next year's potatoes. Experiments on a field scale were started in 1947, and the results now show a useful degree of economic control. The Ayrshire setting which necessitated summer injections therefore allowed a longer period of action for the DD than did the earlier experiments collated by Peters & Fenwick (1949). This longer time for action is now believed to be important, and it may not be without significance that it involves also a longer period in the warmer parts of the year.

The effects of a single application of DD are seen in the first, the second, and sometimes the third, subsequent potato crops. Yield is increased, and the cyst attack upon the roots is diminished. Average values are:

Years after treatment (potatoes grown each year) ...	1	2	3
Percentage increase in yield over control plots	20	10	4
Percentage decrease in cyst attack of the roots, compared with control plots	55	57	65

Cyst attack of the roots is measured as the average number of cysts per inch of root. This is an ecological measurement which assesses the disease-producing power of the cyst population in the soil, which can hardly be estimated in any other way.

Consecutive annual applications have an even more potent effect:

Crop grown after treatment no.	1	2	3
Percentage increase in yield over control plots	20	23	32
Percentage decrease in cyst attack of the roots, compared with control plots	55	82	88

DD has been applied at 200, 400 and 800 lb./acre. The highest rate damaged the crop, reduced the yield in the first subsequent year, and did not bring sufficient increase in the second and third years to make up the initial deficit. Increased yields from 200 and 400 lb. treatments are roughly in proportion to these respective doses, being worth, collectively over a period of 3 years, about twice the cost of the DD which induced them.

There can be little doubt that DD stimulates the potato crop directly, but part of the increase in yield must also be due to the control of eelworm attack. This is effected by some mechanism which partially empties the cysts of their contents, in the absence of potatoes, without affecting the overall cyst population to a marked degree. The question then arises as

to whether periods of action longer than 1 year would bring even better results. Single experimental treatments given 2, and even 3, years before the crop, show that they do:

No. of years between one single treatment and crop (crops other than potatoes in between)	1	2	3
Percentage increase in yield over control plots	20	43	30
Percentage decrease in cyst attack of the roots, compared with control plots	55	83	90

The increase (43 %) when treatment is given 2 years before the crop is greater than that (30 %) when given 3 years before. This is believed to reflect the maximum effect of direct stimulation in the second year, and the 30 % increase in the third year is probably due almost entirely to the control of eelworm. Useful support for this view comes from the results of trials with other substances which control eelworm without stimulating the crop. When an experimental control of 90 % of eelworm attack is obtained, the increase in yield is almost 30 %. The effect of DD against potato-root eelworm on late crops has not yet been assessed, but this long-term view of its mode of action should lay down certain basic necessities for its ultimate success.

Much detailed work and investigation has been devoted to the design and production of machines suitable for injection. The Auchincruive gravity system employs main tanks shaped to deliver under all slopes likely to be encountered, with subsidiary constant-level tanks to provide even rates of flow. Injection tines have protective side and sole plates behind the point, to protect the final delivery tube. Delivery is arranged via a free-flow indicator to each point—a most necessary provision to avoid gaps in the work of injection. The liquid overflows through an open tube if a blockage occurs in the delivery. A towed cultivator-type model, a tractor-mounted machine with automatic cut-off, and a plough model, have all proved satisfactory in extensive farm use. For small areas, a small, hand-pulled injector has been made. The farm machines are all designed to replace or attach to existing machines used for normal tillage operations, thus cutting down the costs of application to low levels.

Full details of all the work reported here are given in two recent research bulletins by the author (Grainger, 1951*a*, *b*).

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NICKEL TOXICITY IN PLANTS

By J. G. Hunter and O. Vergnano, *The Macaulay Institute for Soil Research, Aberdeen*

In several small areas of Aberdeenshire, crops are seriously affected by nickel toxicity, resulting from the abnormally high nickel content of the soils. These soils and plants, and certain aspects of nickel toxicity in plants grown under controlled conditions, were examined by the authors, and the more general features of the investigation are described here.

The area on which most of the field work was done is situated near Whitecairns, about 8 miles north of Aberdeen, and is a poorly drained basin about 4 miles long and 2 miles wide

surrounded by low hills. The principal rock type is a dark greenish black massive serpentine, with talc and chlorite veining, outcrops of which are common on the hill. This serpentine predominates in the mixed drift cover from which the soils are derived. The soils belong to the Leslie soil association (Glentworth, 1945–6), and except towards the top of the hill, are in cultivation. Farms in the district are 'marginal'.

Of the crops grown in the area, the oat is most characteristically affected, and provides the best illustration of the abnormality. Examination of oat crops shows great differences in the size of plants of the same age, and a variety of symptoms. In every instance, however, white necrotic tissue is present as longitudinal stripes in the leaves of affected plants, which are also to some degree chlorotic. Other symptoms on these plants are similar to those produced by ordinary nutrient deficiencies, and occur throughout the crops as secondary features associated with local differences in the nutrient status of the soil.

The soils throughout the area vary considerably, but two main types can be distinguished, namely, an acid peat (pH 4·5–5·3) in the basin, and a loam or peaty loam (pH 5·6–6·7) on the hill slopes. The phosphorus and potassium status of both types are low, calcium is satisfactory and magnesium high; the manganese status of the hill-slope soils is low. The most striking feature of the soils is the very high acetic-soluble and exchangeable nickel contents:

	Nickel soluble in 2·5 % acetic acid (p.p.m. in soil)	Exchangeable nickel (p.p.m. in soil)
Basin soils	114–289	26–61
Hill-slope soils	49–403	22–49

These values are very high compared with those usually found in soil—acetic-soluble nickel less than 2 p.p.m. and exchangeable less than 0·2 p.p.m. (Mitchell, 1944, 1945). The nickel content of the fully expanded leaves of the oat plants (10–14 weeks from germination) growing on these soils (17–134 p.p.m. in dry matter from the basin plants and 16–51 p.p.m. from the hill-slope) is also high when compared with the values for plants growing on normal soil, namely, 3 p.p.m. in oat leaves and 0·5–4 p.p.m. in pasture plants, the latter being reported by Mitchell (1944, 1945). Other trace elements were determined in the soils and plants; though in some cases the concentration differed from normal, in no instance could it be considered toxic or deficient. Robinson, Edgington & Byers (1935) and Mitchell (1945) have previously reported infertility in soils containing high concentrations of nickel.

Examination of soils and plants from the area shows that uptake of nickel is proportional to the acetic-soluble or exchangeable nickel contents only in soils of similar pH; it is primarily related (inversely) to the soil pH value, being low where this is high and vice versa.

Examination of plants from the affected area and from sand-culture experiments indicates that the effect of nickel on growth and on the production of specific symptoms is related to the nickel content and nutrient status of the plants. The reduction in plant growth, and the degree of necrotic symptoms produced, are increased when the calcium, magnesium, nitrogen, or potassium supply is low or when the phosphorus supply is high; the nickel content of the plants is usually unrelated to the major-nutrient supply, though it may be reduced when this is high.

For practical purposes the most satisfactory corrective treatment for nickel toxicity is the application of lime together with nitrogenous and potassic fertilizers, application of phosphate being restricted as far as is consistent with the prevention of phosphorus deficiency. Where the pH of the soils is already high, lime should not be applied as manganese deficiency may be produced.

The effectiveness of calcium carbonate in preventing or reducing the severity of nickel toxicity is due to the reduction in availability of soil nickel by the increase in soil pH; the acetic-soluble nickel content of the soil, which is unsatisfactory as a measure of nickel availability, is not necessarily altered by the calcium carbonate applications. This was clearly

shown in a pot experiment in which basin soil was mixed with pure quartz sand in the proportion 7 : 2, a low level of nutrients supplied (N 5·04, P 2·37, and K 5·85 mg. 100 g. of mixture), and oat plants grown in (*a*) the mixture, (*b*) the mixture to which 1 g. calcium carbonate was added per 100 g., and (*c*) the mixture to which calcium as sulphate was added in an amount equivalent to the calcium carbonate supplied in (*b*); the experiment was examined 36 days after germination of the seed, and the results obtained are shown in Table 1.

TABLE 1

	Mixture alone	Mixture + $CaCO_3$	Mixture + $CaSO_4$
Mixture pH before experiment	4·8	4·8	4·8
Mixture pH after experiment	4·8	6·3	4·6
Nickel soluble in 2·5 % acetic acid (p.p.m. in mixture)	218	219	204
Degree of necrotic symptoms	High	Nil	High
Nickel (p.p.m.) in fully expanded leaf dry matter	147	41	130

The effect of pH on nickel uptake by plants growing in soil is entirely different to that produced in water culture where considerably more nickel is absorbed at high pH levels than at low.

Throughout the investigation, the production of two types of symptoms by nickel, namely, necrosis and chlorosis, was evident, the latter being due to induced iron deficiency; similar results, obtained from examination of a variety of trace elements, have been reported by other authors (Gile, 1916; Wallace & Hewitt, 1946; Hewitt, 1946, 1947, 1948*a–c*; Millikan, 1947*a*, *b*; Berger & Gerloff, 1947; Morris & Pierre, 1949; Nicholas & Forster, 1950). Experiments, which will be reported elsewhere, showed that a high-level copper supply prevents the appearance of necrotic symptoms, indicating that a nickel-copper relationship may be involved in their production. Millikan (1947*b*) has suggested that high-level molybdenum will alleviate the chlorosis produced by nickel in flax, but this was not confirmed in experiments carried out by the authors with oats or by Hewitt (1948*c*) with sugar beet.

Two oat crops (one affected to a moderate degree by nickel toxicity, the other severely affected) growing in the basin were examined at intervals throughout the season, nickel being determined in the dry matter of selected parts. The results are given in Table 2, and though some irregularity is apparent, several general trends in the distribution of nickel can be distinguished, namely, that considerable concentrations of nickel occurred in all parts of the plant but that the leaves usually contained more than the stems, the young parts more than the old, the leaf blades more than the leaf sheaths, the flowers more than the peduncles, and the grain more than the straw.

The effect of nickel toxicity on a range of crops was examined by field experiments on the basin soil, comparison being made between plants from (*a*) untreated plots, (*b*) plots which received 2 cwt. potato fertilizer* and 2 tons of hydrated lime per acre, and (*c*) plots which received three times the amount of fertilizer and twice the amount of lime given in (*b*). Plant analyses indicated the presence of several major-element deficiencies, especially in the (*a*) plots, but symptoms not associated with these (and confirmed in sand-culture experiments as being due to nickel) were also present except in bean (variety, Tick) and are described in Table 3. Of the crops examined, yield data showed that barley was the least affected and that wheat, rye-grass and bean were little affected. The other crops were all much affected, but with applications of lime and fertilizers (especially at the higher level) growth of oat, clover and potato was good and that of turnip (yellow and swede), cabbage and kale much improved. Beet (fodder type) was the crop most affected, and growth was

* Containing 6·5 % N, 6·5 % soluble P_2O_5, 4 % insoluble P_2O_5, 12 % K_2O.

little improved even with the high lime and fertilizer treatment; in this connexion it may be mentioned that Hewitt (1948*c*) has drawn attention to the extreme susceptibility of beet to nickel toxicity. The nickel content of selected parts of the plants was determined (Table 4),

TABLE 2. *Distribution of nickel in oat plants* (*variety*: *Victory*)

	Nickel concentration (p.p.m.) in dry matter	
	Moderately affected plants	Severely affected plants
Young plants—70 days after sowing:		
Leaves	35	109
Stems	42	60
Pre-flowering plants—93 days after sowing:		
Old leaf blades	24	—
Fully expanded leaf blades	54	—
Young leaf blades	42	—
Lower third of stems + leaf sheaths	25	—
Middle third of stems + leaf sheaths	39	—
Upper third of stems + leaf sheaths	35	—
Flowering plants—111 days after sowing:		
Old leaf blades	57	199
Fully expanded leaf blades	54	162
Young leaf blades	87	159
Old leaf sheaths	21	83
Fully expanded leaf sheaths	16	81
Young leaf sheaths	28	101
Lower third of stems	18	36
Middle third of stems	22	68
Upper third of stems	74	168
Flowers	89	151
Peduncles	32	108
Mature plants—170 days after sowing:		
Straw	37	99
Grain	73	141

TABLE 3. *Nickel toxicity symptoms*

Crop and Variety	
Oat (Victory), wheat (Fylgia), rye-grass (Italian)	Leaves chlorotic and necrosis present as colourless longitudinal stripes
Barley (Ymer)	Similar to oat, but leaf tips normally necrotic. Necrotic areas initially colourless but usually become brown
Clover (Broad-leaved Red)	Chlorosis in first leaves as fine pattern and whole leaflets occasionally completely necrotic (colourless)
Turnip, yellow (Balmoral), swede (Balmoral)	First leaves chlorotic, older leaves yellow or brownish yellow. Scattered brown necrotic areas present
Potato (Kerr's Pink)	Few leaves chlorotic and ultimately yellow with scattered brown necrotic areas
Beet (Pajbjerg Rex)	Leaves yellowish green with scattered brown necrotic areas
Cabbage (McEwan's Early), kale (Thousand-headed)	Leaves chlorotic or yellow with scattered brown necrotic areas

and it was found that barley and wheat accumulated little nickel whilst bean accumulated large amounts. Information on the distribution of nickel in various parts of the plants, and change in concentration with age, is available from the results in Table 4: in oat, barley and wheat, nickel accumulated in the leaves and seeds; the concentration of nickel was greater

in the leaves of rye-grass than in a sample made up of stem + leaves + seeds; in clover, nickel accumulated in the leaves; in turnips (yellow and swede), only small concentrations were found in the bulbs, the leaf laminae containing higher concentrations particularly early in the season; the nickel content of potato tubers was low, whilst it was high in the leaf laminae; in cabbage, the leaf laminae contained more nickel than the midribs; high concentrations occurred in both the leaves and seeds of bean, the concentration in the former being greater than in the latter. Lime + fertilizer application markedly reduced the degree of nickel toxicity symptoms, and usually reduced the nickel content of the plants, the reductions being due to the more satisfactory nutrient status of the plants and to the reduced availability of soil nickel; the soil pH values in the plots were (*a*) 4·8, (*b*) 5·3 and (*c*) 6·0.

TABLE 4. *Nickel content of plants*

Crop	Sampling stage	Part analysed	Nickel concentration (p.p.m.) in dry matter		
			O*	M*	H*
Oat	Mature	Fully expanded leaves	42	43	33
		Straw	26	24	16
		Grain	47	54	50
Barley	Mature	Straw	4	4	2
		Grain	7	8	6
Wheat	Mature	Fully expanded leaves	4	3	5
		Straw	3	3	4
		Grain	17	14	11
Rye-grass	Seeding	Fully expanded leaves	48	43	40
		Complete plants (except roots)	39	34	32
Clover	Flowering	Leaves	58	48	35
		Flowers (and peduncles)	26	22	37
Turnip, yellow	Bulbs beginning to expand	Fully expanded leaf laminae	99	74	71
	Mature	Fully expanded leaf laminae	—	57	34
		Bulbs	—	23	26
Turnip, swede	Bulbs beginning to expand	Fully expanded leaf laminae	73	77	44
	Mature	Fully expanded leaf laminae	—	39	27
		Bulbs	—	20	18
Potato	Flowering	Fully expanded leaf laminae	35	33	33
	Mature	Tubers	7	7	7
Beet	Bulbs beginning to expand	Fully expanded leaf laminae	—	75	48
Cabbage	Mature	Fully expanded leaf laminae	—	32	28
		Fully expanded leaf midribs	—	15	14
Kale	Mature	Fully expanded leaf laminae	—	46	33
Bean	Mature	Fully expanded leaf laminae	90	78	50
		Seeds	59	58	51

* O = nil treatment; M = moderate level of N, P, K and lime supplied; H = high level of N, P, K and lime supplied.

The yield from some nil treatments was insufficient for analysis.

Examination of serpentine soils from other areas indicated that though the trace-element concentration in some may be non-toxic, the concentration of nickel or chromium in others may be sufficiently high to produce toxicity. The diagnosis of trace-element toxicities in soils is most satisfactorily made by pot experiments in which symptoms are observed and the trace-element content of the plant leaves determined; the oat plant is a suitable indicator

because it is relatively susceptible, and characteristic symptoms are produced on it; where nickel toxicity is involved, symptoms are quite distinct from those of copper, chromium, zinc, molybdenum, manganese and boron, but may be confused with those of cobalt, though they are not identical; however, where cobalt is toxic, the cobalt concentration in the leaves is high, being greater than 50 p.p.m. in the dry matter.

The nickel analyses in this paper were supplied by Dr R. L. Mitchell, Department of Spectrochemistry, The Macaulay Institute for Soil Research, to whom the authors are greatly indebted.

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REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1951

The Officers and Council of the Association were as follows:

President: R. W. Marsh, M.A.
Vice-Presidents: J. W. Evans, M.A., Sc.D.; H. C. Gough, B.Sc., Ph.D.
Hon. Treasurer: H. F. Barnes, M.A., Ph.D.
Hon. Gen. Secretary: R. K. S. Wood, B.Sc., Ph.D. (on leave of absence September 1950–November 1951 inclusive).
Hon. Programme Secretary: L. Broadbent, B.Sc., Ph.D. (also acting General Secretary September 1950–November 1951 inclusive).
Hon. Editor of the *Annals of Applied Biology*: R. W. Marsh, M.A.
Hon. Asst. Editor: I. Thomas, M.Sc., Ph.D.
Council: Prof. J. B. Cragg, M.Sc.; J. W. Evans, M.A., Sc.D.; C. E. Foister, B.A., Ph.D.; Miss M. T. Franklin, B.Sc., Ph.D.; S. D. Garrett, M.A., Sc.D.; H. C. Gough, B.Sc., Ph.D.; R. M. Greenslade, B.Sc., Ph.D.; R. V. Harris, D.Sc.; I. Isaac, B.Sc., Ph.D.; A. B. P. Page, D.Sc.; I. W. Prentice, M.Sc., Ph.D.; Mrs M. A. Watson, B.Sc., Ph.D.

During 1951 sixty new members were elected. This number was exceeded only in 1947 when sixty-two were elected.

The names and addresses of all members are included in the List of Members published in Vol. **38**, Part 4, with the exception of those elected in December. These are:

Davies, T. G., M.Sc., N.A.A.S., Bryn Adda, Penrhos Road, Bangor, N. Wales.
Goodchild, N. A., B.Sc., The Tea Research Institute of East Africa, P.O. Box 91, Kericho, Kenya.
Heathcote, G. D., B.Sc., Rothamsted Experimental Station, Harpenden, Herts.
Singh, B. P., M.Sc., Rothamsted Experimental Station, Harpenden, Herts.
Usher, G., B.Sc., 27 Park Wern Road, Sketty, Swansea.

The following changes of address were received too late for inclusion in the List:

I. F. Storey *to* N.A.A.S., Anstey Hall, Trumpington, Cambridge.
P. Bracey *to* Colonial Office Research Unit, C.D.E.E., Porton, Salisbury, Wilts.
M. Burton *to* 55 Popes Grove, Twickenham, Middlesex.
R. H. E. Bradley *to* Box 971, Fredericton, N.B., Canada.
M. Hafez *to* Entomology Department, Faculty of Science, Fouad I University, University Post Office, Giza, Egypt.
A. R. Loveless *to* Department of Botany, University College of the West Indies, Mona, St Andrew, Jamaica, B.W.I.

The following four resignations were accepted during the year: A. E. Cameron, Mrs D. Bone, J. Rhodes and Mrs M. E. Wilson. On 31 December 1951, membership stood at 606, but the following nine members resigned from the end of the year: F. C. Atkins, Miss E. M. Blackwell, J. A. Downes, C. H. Gadd, Dame Helen Gwynne-Vaughan, E. M. Hutton, Mrs M. J. Reavill, I. C. Smith and G. T. Spinks. The following two members are presumed to have resigned: A. H. R. Lilly and P. Satyanarayana.

Honorary Membership of the Association was conferred during the year on Mr C. Warburton, the Association's only remaining Original Member.

The Council regrets to record the deaths during the year of Prof. I. Tragårdh (Hon. Member 1949), the distinguished Swedish forest entomologist; Mr G. Fox Wilson (1920),

the President, whose lamented death before the expiry of his term of office was briefly mentioned in Council's report for 1950; Dr F. A. Baker (1943), of the Rowett Research Institute; Dr G. H. Bates (1938), the Director of the Penkridge Farm Institute; and Mr Paul Jefferies (1945) as a result of a street accident.

Six meetings were held in London and one at Wye with an average attendance of seventy members and thirty-eight visitors. The programmes included the following:

9 *February*. Films: The medical aspects of venomous snakes. Recherches expérimentales sur les champignons prédateurs des nematodes du sol (introduced by Dr T. Goodey). Insect pests in food (introduced by Dr Parkin). Improving your raspberry crop (introduced by Dr Cadman).

16 *March*. Symposium on birds and rodents in relation to agriculture. A. ROEBUCK: Observations on the woodmouse. D. CHITTY: Rats, mice and agriculture. H. V. THOMPSON: Rabbits and other rodents. J. W. EVANS: Birds and agriculture. Films: The rural rat and The rabbit pest.

18 *April*. J. B. CRAGG and Mrs M. VINCENT: Action of metaldehyde on slugs. D. W. FENWICK: Problems in the bio-assay of potato root diffusates. R. H. DAVIS: Stored products insect control in the Tropics of S.E. Asia, the Middle East and Central America. A. H. M. KIRBY: The chemical control of the apple sawfly. J. W. BLENCOWE: Effects of time of sowing and singling sugar beet on yellows virus infection. P. H. GREGORY: Wind tunnel experiments on particle deposition.

26 *September*. R. L. WAIN: Research on plant growth regulating substances at Wye. H. W. MILES: Work in the Horticultural Department at Wye. Miss A. B. BEAKBANE: The growth and behaviour of scion and stock in relation to their anatomy. A. F. POSNETTE: Recent research at East Malling on virus diseases of fruit trees.

12 *October*. Symposium on insect physiology in relation to applied biology. Introduction by the Chairman, V. B. WIGGLESWORTH. W. A. L. DAVID: Insects in relation to the accumulation and adherence of insecticides. J. W. L. BEAMENT: The role of cuticle and egg membranes in the penetration of toxic materials. C. POTTER, K. A. LORD, and A. H. MCINTOSH: Studies on the physical and biochemical action of insecticides. A. MILNE: Features of the ecology and control of the sheep tick. J. ALLAN CAMPBELL: The developmental cycle of the sheep tick. A. D. LEES: Aspects of the physiology of the sheep tick.

7 *November*. Symposium on photography in applied biology. H. L. NIXON: Electron microscopy. J. SMILES: Microscopy and photomicrography. W. H. WALTON: The flash photography of flying insects (mosquitoes). D. L. CULLEN: Photographic techniques, colour materials and printing papers. J. A. WALKEY: The preparation and reproduction of scientific illustrations for publication. S. BEAUFOY: Insect photography.

10 *December*. T. SWARBRICK: The use of fixed-wing aircraft for crop spraying. F. RAW: The ecology of the garden chafer (*Phyllopertha horticola* (L)). A. F. POSNETTE: Cacao selection in the Gold Coast.

By kind permission of the Directors of Imperial Chemical Industries Ltd., the Spring Meeting was held on 1 June 1951 at their Butterwick Research Laboratories, Welwyn. Some seventy-five members and guests visited the various departments and were entertained to luncheon and to tea. The Council wish to record their deep appreciation of the work done by Dr P. W. Brian and his colleagues in arranging this successful and interesting meeting.

A Conversazione was held at the Imperial College, 2.30–6.00 p.m. on 27 July. Some two hundred people attended, eighty staying to supper at its conclusion. The large number of exhibits was divided as follows. Virus diseases of plants and insects—5. General and agricultural mycology—5. Nematode diseases of plants—4. General and applied entomology—5. Plant nutrition—3. Agricultural zoology—3. Weed-killers and antibiotics—3. Miscellaneous—2. Instruments and apparatus for biological research—11. The Council wish to record its thanks to Mrs Marion Watson who was responsible for the arrangements in connexion with this meeting.

The Summer Meeting was held at Wye College and East Malling Research Station on 24–28 September. Mr B. Furneaux and Mr A. G. Davies gave talks about Romney Marsh during the evening of 24 September. On 25 September some forty-six members and guests went on a tour of agricultural areas of East Kent. A paper-reading session held during the morning of 26 September was attended by eighty-four members and guests, and during the afternoon demonstration tours were made of the departments of Chemistry, Geology, Botany, Zoology and Plant Pathology. On 27 September members and guests visited East Malling Research Station. After being welcomed by the Director, Dr Tubbs, the party visited the plantations and laboratories. During the evening the Principal of Wye College, Dr Skilbeck, gave a reception and dinner. The meeting concluded with a luncheon at East Malling Research Station on 28 September, which was preceded by visits to two fruit farms in the vicinity. The Council wishes again to record their thanks to Dr Tubbs and Dr Skilbeck for their kindness in receiving members of the Association and for their generous hospitality. The arrangements for this meeting were made by Miss Schimmer, acting as Local Secretary, by Dr Harris, Mr Glasscock, and their colleagues.

The Council record with pleasure the honours conferred by his late Majesty, King George VI on the following members during the year: Dr W. J. Hall, C.M.G., and Dr V. B. Wigglesworth, C.B.E.

The Council wish to thank Dr L. Broadbent for acting as Honorary General Secretary in the absence of Dr R. K. S. Wood.

The Association was represented on the Parliamentary and Scientific Committee, the Biological Council and the National Committee for Biology. Two members served as delegates to the 9th International Entomological Congress in the Netherlands.

During the year application was made to the Royal Society for facilities in the proposed Science Centre.

In conclusion, the Council wish again to thank the authorities of the Imperial College for their kindness in providing lecture theatres for meetings and in permitting the use of their catering facilities.

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDING 31 DECEMBER 1951

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £1797. 5*s*. 6*d*., an increase of £146. 17*s*. 3*d*. over the sale of the *Annals* in 1950. The income from the sale of back volumes and parts was £217. 1*s*. 0*d*., an increase of £99. 19*s*. 9*d*., while the sale of reprints and advertisements showed an increase of £118. 6*s*. 8*d*. The cost of production was £3778. 17*s*. 2*d*., an increase of £1187. 16*s*. 11*d*. This was accounted for by the increased size of the volume and the increased costs of production. The net cost of the *Annals* therefore was £1541 as against £673 in 1950.

The amount received during the year for subscriptions and entrance fees, including arrears, was £764. 16*s*. 6*d*., an increase of £55. 12*s*. 0*d*.

Expenditure for the year showed an excess of £892. 3*s*. 10*d*. over income, as against £98. 1*s*. 10*d*. in the previous year. This is accounted for by the greatly increased deficit on the *Annals*.

The Association once again received a grant of £200 from the Royal Society in aid of the *Annals*. This grant is gratefully acknowledged.

The net assets of the Association at the end of the year amounted to £2451. 0*s*. 10*d*., a decrease of £692. 3*s*. 10*d*. All available surplus funds are invested in National Savings Certificates (£1774. 3*s*. 9*d*.) and 2½% Defence Bonds (£400. 0*s*. 0*d*.). The estimated value of the stock of the *Annals of Applied Biology* is £164. 12*s*. 0*d*.

REPORT OF THE HONORARY EDITOR FOR 1951

The increase authorized by Council in the size of vol. **38** of *The Annals of Applied Biology* has reduced the period between receipt and publication of papers to approximately 8 months. Another sharp rise in printing costs will however necessitate some reduction in the size of the 1952 volume.

Vol. **38** contained xv + 929 pages and 25 Plates (one in colour) as compared with xii + 707 pages and 14 Plates for vol. **37**. Including papers published in *Proceedings*, the 1951 volume contained 87 communications (26 more than in 1950), the subjects being roughly classified as follows: general applied botany, 11; mycology and plant diseases, 17; viruses and virus diseases, 13; entomology and insect pests, 19; insecticides and fungicides, 12; plant nematodes, 6; general applied zoology, 9. There were 14 papers by non-members, of which 10 were *Proceedings* papers.

At the end of 1951 the printing order was raised from 1450 to 1700 copies.

The dates of issue of the four parts were: no. 1, 31 March; no. 2, 2 July; no. 3, 3 October; no. 4, 3 January.

The two vacancies on the Publications Committee were filled by the appointments of Mr F. J. Anscombe and Dr J. T. Martin. This Committee and the Assistant Editor have shouldered unusually heavy burdens in the past year: these services to the Editor and to the Association as a whole are recorded with gratitude.

ASSOCIATION OF APPLIED BIOLOGISTS PLANT PESTS AND DISEASES COMMITTEE, REPORT FOR 1951

Two Meetings of the Committee have been held during the year, on 7 November and 6 February. J. F. H. Cronshey, J. B. Goodey, N. F. Robertson, L. E. W. Stone and Miss G. M. Waterhouse were appointed and approved by Council to fill the vacancies caused by the retirement under the rules of W. P. K. Findlay, C. G. Johnson and A. R. Wilson. At the first meeting H. E. Croxall was re-elected Chairman and E. Dunn, Secretary.

The Sub-Committee formed, in collaboration with the Ministry of Agriculture Conference of Advisory Entomologists, has completed its work in the compilation of Part II of the list of common and scientific names of British Insect and other pests. It is hoped to publish this second part shortly.

The Sub-Committee appointed to collect information relating to the susceptibility of fruit varieties to damage by spray materials is at present preparing a scheme for the collating of reports of such damage from Research Stations, Advisory Workers and Horticulturalists.

The work of the Sub-Committee appointed to prepare a list of definitions of terms used in Economic Entomology has been held up owing to the weakening of the Committee by the death of Mr Fox-Wilson. The Committee has instructed the Sub-Committee to appoint a new member to fill the vacancy.

After some deliberation the Committee decided that it was unnecessary to hold a 2nd Revision Course in Nematology for at least another 3–4 years. The Committee again stressed the need for greater interest in this subject by the Universities and Colleges, in view of the economic importance of plant nematodes.

The Committee considered that crop observation plots should be established at suitable centres for the study of natural infestation by pests and diseases, and have recommended to Council that a symposium entitled 'Survey of progress of Pest and Disease Assessment' be held in the future. It was also suggested that members of the Association at Research Stations, Agricultural Colleges and Agricultural Departments of Universities might co-operate with advantage in the quantitative survey work now being promoted by both the Disease and Pest Committee of the N.A.A.S. and the Plant Pathology Laboratory, Harpenden.

REVIEWS

A Textbook of General Physiology. By HUGH DAVSON, D.Sc. Pp. 659. London: J. and A. Churchill Ltd. 45*s*.

For those of us who used Bayliss's *Principles of General Physiology* as our guide and inspiration, a new book on General Physiology must raise mixed feelings. On the one hand it will be welcomed because a quarter of a century of discovery has passed since the last edition of Bayliss. On the other hand, we cannot help but wonder whether it is within the compass of one man to cover the extensive and highly specialized fields which now make up the bulk of General Physiology. Dr Davson deserves our congratulations for his courage in tackling this very difficult project, and our praise for the manner in which he has carried out his work.

The book is arranged in six parts. Part 1 deals with the structural basis of living matter, and includes an account of the various methods now in use for revealing microscopic and sub-microscopic structure. It ends with a useful summary of modern work on plant cell walls, arthropod cuticle and annelid cuticle. Part 2 describes the transformations of energy in living systems, careful consideration being given to the problems of heat production and heat loss in animals. Part 3, which forms practically one-third of the book, ranges widely over the various aspects of water and solute transport. Beginning with a general study of osmotic phenomena, it discusses the structure and properties of the plasma membrane, the cerebrospinal fluid, absorption from the intestine, acid secretion by the stomach, kidney function and the complex problems of ionic equilibria and bioelectrical potentials. The characteristics of excitable tissue are dealt with in Part 4. This section summarizes with great clarity the many additions to our knowledge of nerve and sense organ physiology which have been made in recent years. Part 5 covers the mechanism of muscular contraction. Electron-microscope and X-ray studies are described as well as the usual mechanical and chemical changes occurring during muscle activity. The final part has as its title 'Light: Its Effect on, and its Emission by, the Organism'. Here, in ninety pages, is an account of photosynthesis, the effect of light on animals and the biophysics and biochemistry of light production in animals.

The book contains a large number of excellent illustrations, and its fifteen plates, many of them electron-micrographs, deserve special praise. The index is adequate but it is a pity that it has not been possible to include an authors' index.

Obviously, it is difficult in a book of this size to please everybody. Several important aspects of general physiology are not included, and the author names various subjects which were written up but had to be left out through lack of space. Many will join the reviewer in hoping that when another edition appears the section on the comparative physiology of respiration which 'died at conception' will arise from the dead, for a modern book on general physiology is hardly complete without it.

All biologists and would-be biologists should find something of use to them in this book. Britain has never been short of good text-books on physiology, but of late their emphasis has naturally become more and more concerned with mammalian physiology. Dr Davson's book by its high standards and general approach goes a long way towards redressing the balance.

J. B. CRAGG

Die Larvenformen der Dipteren. 2. Teil. By W. HENNIG. Pp. 458. Berlin: Akademie-Verlag. 1950. 49 DM.

The second part of this work is a continuation of a description of the larvae of the suborder Nematocera. Whereas Part 1 dealt with the Bibliomorpha, the larvae of which are predominantly terrestrial, Part 2 describes the larvae of the Culicomorpha, most of the larvae of which are aquatic.

The Culicomorpha are divided into the Psychodiformia, Culiciformia and Tipuliformia and the volume is large because the families dealt with are of great economic importance and many are described in considerable detail. The Psychodiformia and Culiciformia, particularly the latter, contains many families of medical significance; the family Culicidae alone, of which there are some 2700 known species and 1400 known larvae, occupies 135 pages. The remaining families in the Culiciformia—the well-known Simulidae, Chironomidae and Ceratopagonidae appear as Melusinidae, Tendipedidae and Heleidae respectively, names which will be quite unfamiliar to the general entomologist in this country.

The Tipulidae are dealt with in some twenty pages, and since it is possible in a study of this nature to give keys only as far as genera the work will not prove to be of great importance to the agricultural entomologist. On the other hand, from the point of view of the student of Diptera, it will be indispensable; there are 236 excellent line drawings and references are given to the actual pages of books and papers containing descriptions of the genera dealt with.

It is unfortunate that this very important contribution to the literature on this subject should appear in very flimsy paper covers and uncut.

I. THOMAS

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1951 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To Estimated Value of Stock, 1 January 1951		181 10 0	By Sales—Current volume	1797 5 6	
To Cambridge University Press:			Back volumes, Parts and Sets	217 1 0	
Paper and Printing	2780 14 0		Reprints and Advertisements	240 12 5	
Binding	244 13 3				2254 18 11
Carriage	116 17 11		By Estimated value of Stock at 31 December 1951		164 12 0
Insurance and Sundries	11 14 2		By Balance carried down		1540 16 3
Commissions	624 17 10				
		3778 17 2			
		£3960 7 2			£3960 7 2

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1951 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		1540 16 3	By Members' Subscriptions:		
To Printing and Stationery		84 6 3	Arrears	22 10 0	
To Postages and Cheque stamps		42 0 4	Entrance Fees	36 4 0	
To Subscription—Parliamentary Science Committee		7 7 0	Current	706 2 6	
To Sundry Out-of-pocket expenses of Editors, Secretaries and Treasurer		9 1 9			764 16 6
To Refreshments		19 5 3	By Sundry Income—Spring Meeting	5 15 0	
To Hire of Rooms		8 16 0	Summer Meeting	134 15 8	
To Audit Fee		7 7 0	Conversazione	16 16 0	
To Spring Meeting	6 0 0				157 6 8
To Summer Meeting	134 15 8		By Net receipts—*List of Common Names*		1 10 0
To Conversazione	30 17 6		By Interest received and receivable:		
		171 13 2	2½% Defence Bonds	10 0 0	
			National Savings Certificates	32 10 6	
			Post Office Savings Bank	32 5 6	
					74 16 0
			By Balance, being excess of Expenditure over Income for the year		892 3 10
		£1890 13 0			£1890 13 0

Dr. BALANCE SHEET AT 31 DECEMBER 1951 *Cr.*

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors:			Cash:		
Cambridge University Press	1663 18 3		Westminster Bank: Current Account	659 3 11	
Audit Fee	7 7 0		Post Office Savings Bank Account	1323 17 3	
		1671 5 3			1983 1 2
Subscriptions and Entrance Fees paid in advance		24 10 10	2000 National Savings Certificates (Cost £1265 1s. 0d.)		1774 3 9
Life Membership Fund Reserve		175 0 0	2½% Defence Bonds		400 0 0
Excess of Assets over Liabilities:			Stock of *Annals of Applied Biology* as estimated		164 12 0
As per Balance Sheet 31 December 1950	3143 4 8				
Add: Royal Society Grant	200 0 0				
	3343 4 8				
Less: Balance of Income and Expenditure Account for 1951	892 3 10				
		2451 0 10			
		£4321 16 11			£4321 16 11

H. F. BARNES, *Hon. Treasurer*

I certify that the foregoing accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and in my opinion the Balance Sheet exhibits a true and correct view of the state of affairs of the Association according to such books, vouchers and documents.

(Signed) J. B. BENNETT *Chartered Accountant* } *Auditor*

HARPENDEN, 22 February 1952